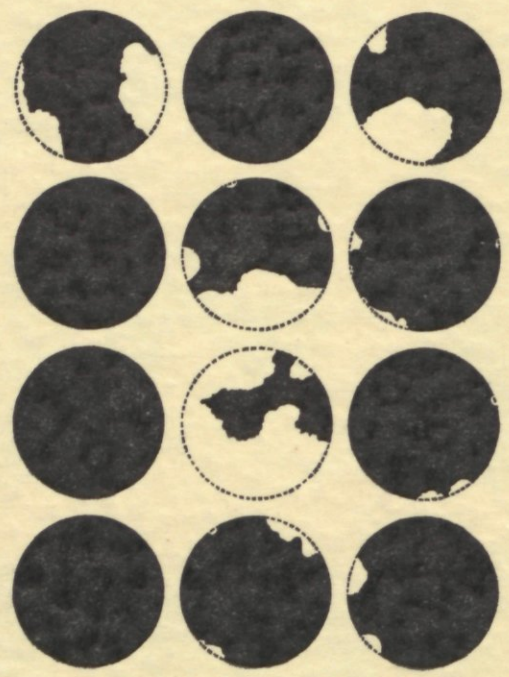


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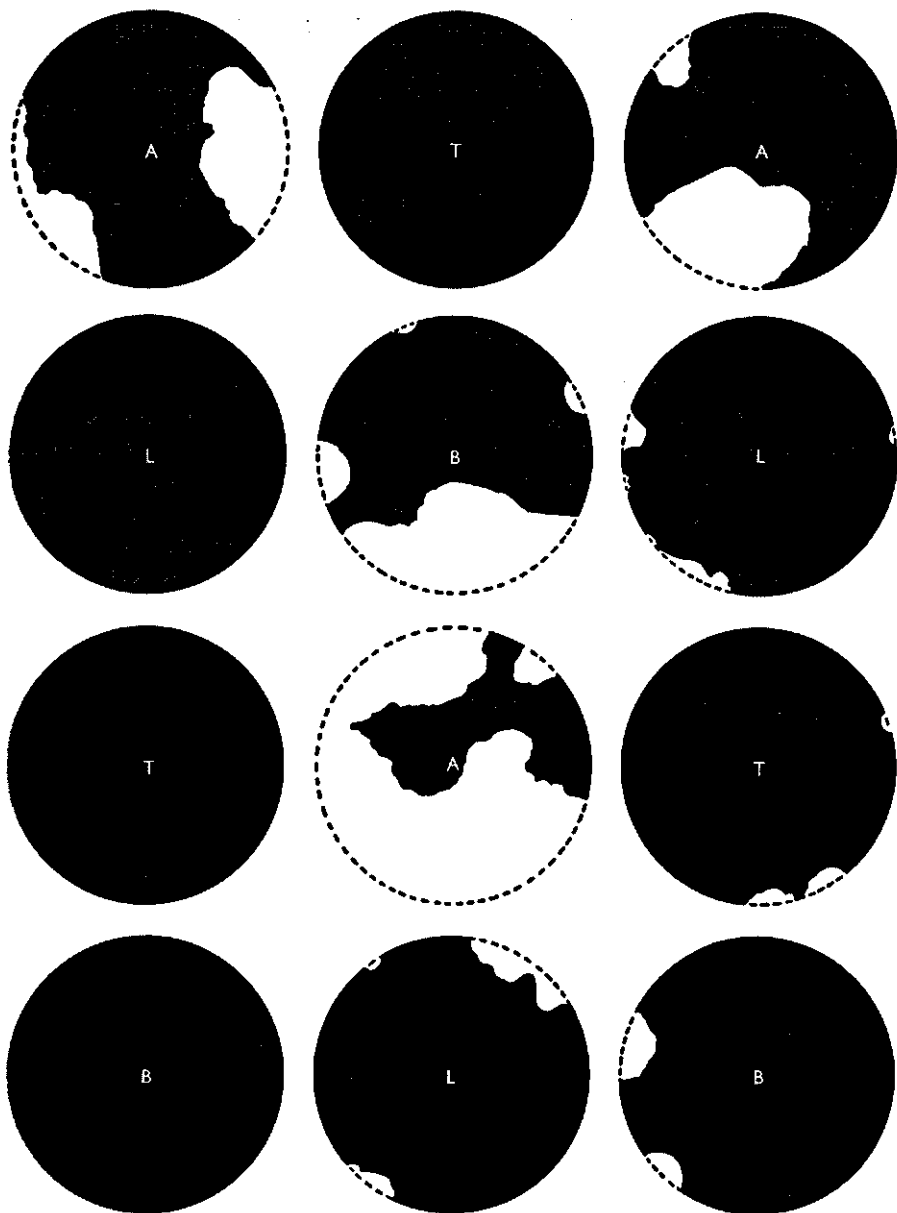
ASPECTS OF HOST-PLANT RELATIONSHIP
OF THE COLORADO BEETLE



W. BONGERS

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Feeding from discs of 4 different plant species, offered according the scheme of fig. 2 (1) page 16, by 6 larvae, reared on *Solanum tuberosum* (diameter of the discs: 2 cm).

ASPECTS OF HOST-PLANT RELATIONSHIP OF THE COLORADO BEETLE

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS, DR. IR. F. HELLINGA,
HOGLERAAR IN DE CULTUURTECHNIEK,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP DONDERDAG 11 JUNI 1970 TE 16.00 UUR

DOOR

W. BONGERS

Dit proefschrift met stellingen van

WILLEM BONGERS,

doctorandus in de wiskunde en natuurwetenschappen, geboren te Bandoeng, Indonesië, op 15 november 1932, is goedgekeurd door de promotor, Dr. J. DE WILDE, hoogleraar in het dierkundig deel van de plantenziektenkunde.

De Rector Magnificus van de Landbouwhogeschool,
F. HELLINGA

Wageningen, 28 April 1970.

This thesis is also published as Mededelingen Landbouwhogeschool Wageningen 70-10 (1970)
(Communications Agricultural University Wageningen, The Netherlands)

STELLINGEN

I

Voedselkeuze en ovipositiekeuze zijn bij de coloradokever niet oorzakelijk verbonden.

Dit proefschrift.

II

De voorkeur voor de waardplant wordt bij de coloradokever niet beïnvloed door prae-imaginale ervaring.

Dit proefschrift.

III

De voedselpreferentie van insecten kan een functie zijn van de temperatuur; deze dient de bij resultaten van proefnemingen daarom expliciet te worden vermeld.

Dit proefschrift.

IV

Het experimenteel onderzoek naar de waardplantrelaties van insecten dient meer door veldonderzoek te worden gesteund dan tot dusver het geval is.

V

De conclusies uit het experimentele werk en de resultaten van het theoretische populatie-dynamische werk van HUFFAKER et al. zijn veeleer de vrucht van "wishful thinking" dan een poging tot causale analyse.

C. B. HUFFAKER, K. P. SHEA & S. G. HERMAN, 1963. *Hilgardia*, 34:305-329.

M. P. HASSEL & C. B. HUFFAKER, 1969. *The Canadian Entomologist*, 101:353-361.

VI

Met de door VAN NIEUWENHOVEN beschreven lage bezoëksfrequentie van de onderzoeker aan grotten, waarin vleermuizen overwinteren, kan de continue slaaperiode van vleermuizen niet worden vastgesteld.

P. N. VAN NIEUWENHOVEN, 1956. *Publ. Nat. Hist. Genootschap*, 9:29-38.

VII

Bij de interne migratie aan het einde van de winterslaapproiode in de groeven van Zuid Limburg, van dieper gelegen gangenstelsels naar het inganggebied van deze groeven, zoeken de vleermuizen de nabijheid van de ingang.

S. DAAN & H. J. WICHERS, 1968. Z.f. Saugetierkunde, 33 (5):262-287.

VIII

Het zoölogisch beheer van natuurreservaten dient niet te worden verpacht aan jagers, maar geheel in handen te blijven van de beherende instanties.

IX

De storingsorganismen, die in een gestoord oecosysteem voorkomen, hebben biologisch gezien, de functie een weg te openen voor nieuwe differentiëring.

X

Het strenger vervolgen en zwaarder straffen van diegenen, die afval wegwerpen op straat, in grachten, parken, bermen, recreatie- en natuurgebieden etc. is niet de juiste weg om dit asociale gedrag tegen te gaan. De oplossing dient te worden gezocht in een grondige integratie van de milieu-educatie in opvoeding en onderwijs.

H. HOESTRA, 1968. Dissertatie, Wageningen, Stelling XI.

XI

Bij een algemeen welzijnsbeleid moet het accent worden verlegd van de individuele naar de collectieve bestedingen.

XII

Het overzichtelijk maken van kruispunten werkt de verkeersveiligheid niet in de hand.

XIII

Met het handhaven van een onlogische spellingswijze, als die van 1954, doen wij onze kinderen onrecht.

*aan Griet
Margreet
Elizabeth
Jan Willem Frederik*

Wie leert, aan verscheiden soorten van
insekten, de eitjes op die bladeren,
welke zij zelve eten, en de jongen
alleen lusten zullen, te leggen!

J. F. MARTINET

Katechismus der Natuur 3:19
Zalt-Bommel, 1828.

VOORWOORD

Het is mij een voorrecht dit proefschrift te beginnen met het verwoorden van enkele gevoelens jegens een aantal mensen, die in mijn leven, gewild of ongewild, een belangrijke invloed hebben gehad. Het is goed te bedenken met hoeveel je in leven en werken te maken hebt gehad en te overdenken, wat dezen voor je betekenen of hebben betekend; wat zij bijgedragen hebben tot je vorming als mens en als bioloog. Hun allen zij hier dank gebracht.

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Zeergeleerde RINGELBERG en zeergeleerde VERHEIJEN, Uw kritische zin en

Uw wijze van werken waren voor mijn vorming als bioloog van grote waarde.

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Wageningen, 26 april 1970.

CONTENTS

1. INTRODUCTION	1
1.1. Scope of investigations.	2
1.2. Food plant range	3
1.3. Host plant selection	4
1.3.1. Orientation towards the host plant	5
1.3.2. Host plant recognition.	6
1.4. Conditioning of host plant preference	7
1.4.1. General	7
1.4.2. Conditioning of host plant preference in <i>Leptinotarsa decemlineata</i>	10
2. DEFINITIONS AND CRITERIA	11
3. METHODS AND MATERIALS	13
3.1. Experimental insects	13
3.2. Plants	13
3.3. Breeding experiments	14
3.3.1. The suitability of the plant as food substratum	14
3.3.2. Alternate feeding	14
3.4. Selection of food	15
3.4.1. Food choice and food preference	15
3.4.2. Evaluation of the food consumption	17
3.4.3. 'Sandwich'-test	19
3.4.4. Food choice experiments at different temperatures	20
3.4.5. Food choice experiments with impregnated leaves	20
3.4.5.1. Impregnation with decoction or filtrate	20
3.4.5.2. Impregnation with leaf juice	21
3.5. Oviposition	22
3.5.1. Selection of the host plant for oviposition	22
3.5.2. Oviposition choice at different temperatures	22
3.6. Host plant finding.	22
3.6.1. Screen test	22
3.6.2. Olfactometer test	24
4. EXPERIMENTAL SET UP AND RESULTS	25
4.1. The suitability of the plant as feeding substratum	25
4.2. Alternate feeding	25
4.3. Food choice experiments	27
4.3.1. Conditioning to the host plant	27
4.3.2. Further choice experiments with <i>Solanum dulcamara</i>	27
4.3.2.1. Food choice experiments at different temperatures with <i>Solanum dulcamara</i>	32
4.3.2.2. Conclusion.	34
4.3.2.3. Influence of temperature on the preference for food.	34
4.3.2.4. Factors influencing the results.	35
4.3.2.4.1. Inbreeding on <i>Solanum dulcamara</i>	36
4.3.2.4.2. Two cultivars of <i>Solanum tuberosum</i>	37
4.3.3. Choice experiments with <i>Solanum carolinense</i>	37
4.3.4. Choice experiments with <i>Solanum rostratum</i>	37
4.3.5. Choice experiments with <i>Solanum lycopersicum</i>	38
4.3.6. Choice experiments with <i>Solanum luteum</i>	38
4.3.6.1. The 'toxicity' of <i>Solanum luteum</i>	38

4.3.6.1.1.	Food choice experiments with impregnated leaves	39
4.3.6.1.2.	'Sandwich'-test	43
4.3.7.	The experimental arrangement	43
4.3.7.1.	Observation of the food intake at different temperatures during 24 hours	43
4.3.7.2.	Observations on behaviour during the food choice experiments	44
4.3.8.	Summary of the results of the experiments on food preference	45
4.4.	Oviposition	46
4.4.1.	Preliminary experiments	46
4.4.2.	Further choice experiments with <i>Solanum dulcamara</i>	47
4.4.3.	Further choice experiments with <i>Solanum luteum</i>	50
4.4.4.	Oviposition and age of foliage; food deprivation	53
4.4.5.	Summary of the results of the experiments on oviposition	58
4.5.	Orientation to the host plant	58
4.5.1.	Screen test	58
4.5.2.	Olfactometer test	61
5.	DISCUSSION AND CONCLUSIONS	62
6.	SUMMARY	65
7.	ACKNOWLEDGEMENTS	67
8.	SAMENVATTING	68
9.	BIBLIOGRAPHY.	71

I. INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* SAY, is of particular interest on account of an apparent shift in its food habits noticed a little more than a century ago, when it was first observed feeding on the foliage of the potato plant (*Solanum tuberosum* L.). However, the food preferences of this beetle are not as distinct as might be inferred from this statement.

The genus *Leptinotarsa*, to which the Colorado beetle belongs, is confined almost entirely to the continent of North and Central America, extending southward to Panama and northward to the northern United States. By far the majority of the genus is found in southern Mexico. The species *Leptinotarsa decemlineata* was described first by THOMAS SAY (1823) who found it in the Rocky Mountains (Missouri, Arkansas). According to TOWER (1906) the Colorado beetle lived in the eastern part of the United States, where it was probably sparsely distributed over the whole area feeding on *Solanum rostratum* DUNAL, an essentially tropical plant. Though doubt exists concerning the original distribution of *Leptinotarsa decemlineata*, TOWER developed a plausible theory concerning the dispersal of this species. According to this theory it is very probable that the movements of Spanish travel and commerce resulted in the dispersal of *Solanum rostratum* into Texas, New Mexico, and parts of Arizona. Its fruits with persistent calyx are well adapted to being transported by large mammals, being armed with long spines, which hook in the hair of cattle and are thus often carried for long distances. It is assumed that herds of bison on the Great Plains have been an efficient agent of dissemination of *Solanum rostratum* into the North of America.

Wherever the foodplant is dispersed the beetles will follow, provided the soil and climate are favourable, and over the area now occupied by *Solanum rostratum*, conditions for survival of the ancestors of the Colorado beetle (according to TOWER: *Leptinotarsa intermedia*) were favourable. Into this region these beetles must have spread gradually, and on the eastern slope of the Rocky Mountains have undergone evolutionary changes from which *Leptinotarsa decemlineata* eventually emerged.

In this habitat it remained practically unchanged until about 1850, when the western extension of human colonization introduced into its habitat a new plant, *Solanum tuberosum*, which proved to be an acceptable food. The original barrier of dispersal, i.e. lack of food plants, was completely removed by this new food, and there lay open towards the east an expanse of territory where optimum conditions for existence were provided. Into this area it began to spread, making itself felt as an economic factor in agriculture, and within twenty years it reached the Atlantic Ocean and became a dreaded pest in the United States, and since 1920, in Europe also (TOWER, 1906; THOMAS, 1952).

1.1. SCOPE OF INVESTIGATIONS

In insects like the Colorado beetle, the foodplant is primarily chosen by the adult female, the offspring being placed on the plant during the act of oviposition (THORSTEINSON, 1960).

The ovipositing beetles normally deposit the egg masses on the leaves of the plants they themselves feed upon, and the 'taste' of the larvae is generally in agreement with that of their parents. Though differences may be found in food preference between the adult and the larva, or even between the different larval instars (TROUVELOT et al., 1933a), the food range of the larva in general reflects that of the adult in this species (CHIN, 1950).

Leptinotarsa decemlineata lives only on some species of plants belonging to the genus *Solanum*. In literature little information is found concerning host plants of the Colorado beetle in the field other than potato. It is well known that larvae are frequently destructive to eggplants (*S. melongena*) in gardens, and that they appear occasionally on tomato (*S. lycopersicum*), ground cherry (*Physalis spp.*) and on certain varieties of cultivated tobacco (*Nicotiana*) that develop a low nicotine content in the leaves (TOWER, 1906, 1918; FEYTAUD, 1936; BRUES, 1940; CHIN, 1950; BUHR, 1956).

The fact that the Colorado beetle is to be found in the field practically only on potato plants gives rise to several questions about the relationship between *Leptinotarsa decemlineata* and its host plant. Within the scope of this discourse the following questions are of importance:

1. Does oviposition take place on other plants and, if so, on which plants and how frequently?
2. Do beetles prefer certain solanaceous plants to others within the food plant range?
3. Are the behaviour patterns governing oviposition choice and food choice innate or determined by experience?
4. Are selection for food and for oviposition subordinate to one another? In other words: does the female deposit its eggs on the preferred food plant or does it eat the plant preferred for oviposition?
5. Do beetles, which have hatched from eggs on a given food plant, and which develop on that plant, show a food and oviposition preference for the same plant?
6. What is the role of external factors, e.g. temperature, physiological condition of the foodplant, in the host plant relationship of the Colorado beetle?

In short: can we modify the host plant preference of the Colorado beetle? We can imagine for example, that a certain habituation, a process of 'learning', is responsible for host plant affinity, as has been suggested by HOPKINS (1916; see also page 7). In other words: that the adult beetle will prefer that plant on which it lived as a larva.

1.2. FOOD PLANT RANGE

Some plants may be eminently acceptable to *Leptinotarsa decemlineata* but still do not belong to the normal range of host plants because their geographical ranges are not coextensive. There also may be plants which grow under such conditions of temperature, humidity, soil characteristics and light as to make their habitat unfavourable for the Colorado beetle. Another factor required for a suitable host plant is coincidence in time: unless the growing season of a plant coincides with the feeding stages of *Leptinotarsa decemlineata*, the plant obviously can not serve as food (DETHIER, 1954a).

Many rearing experiments have been performed and described by several authors, with a view to investigate whether or not the Colorado beetle can be reared for a longer or shorter time on certain plants. Few statistical data are available. With regard to these experiments it must be taken into account that under the threat of impending starvation many insects tend towards wider polyphagy and can survive on an altered diet (BRUES, 1939). Furthermore, even insects with restricted diets can, by various experimental artifices, be induced to feed successfully on other plants (DETHIER, 1947).

It is not our intention to give a detailed survey here of all breeding experiments and a complete list of all accepted or rejected species in the family Solanaceae in reference to the feeding of the Colorado beetle. For detailed accounts concerning food plants see the works of TOWER (1906), BRUES (1940), CHIN (1950), BUHR (1956), and TROUVELOT (1958). The following discussion refers especially to *Solanum tuberosum*, *Solanum dulcamara*, *Solanum nigrum*, *Solanum lycopersicum*, *Solanum rostratum* and *Solanum carolinense*.

TOWER (1906, 1918) mentions *Solanum rostratum* (buffalo bur) as being the original food plant of the Colorado beetle. This species is greedily accepted according to experimental work of BRUES (1940) and the insect can be bred continuously upon it as TROUVELOT et al. (1933a, b), MCINDOO (1935), BUHR (1956) and TROUVELOT (1958) have found.

Since the Colorado beetle is a pest on *Solanum tuberosum*, no comment is needed in reference to this plant.

TROUVELOT et al. (1933a, b), MCINDOO (1935), FEYTAUD (1936), JERMY and SÁRINGER (1955b), BUHR (1956) and TROUVELOT (1958) reared the beetle successfully on *Solanum dulcamara*. BRUES (1940) too states that bittersweet is greedily accepted and voraciously fed on; CHIN (1950) also reports bittersweet to be attractive. The last two authors only state that this plant is accepted, they do not mention breeding experiments. According to JERMY and SÁRINGER (1955b) the larvae take several days longer to mature on this plant than on potato.

TOWER (1906) quoted *Solanum carolinense* (horse nettle) only as a food plant for a related species (viz. *Leptinotarsa juncta* GUER.) but not for *Leptinotarsa decemlineata*. MCINDOO (1935), BUHR (1956) and TROUVELOT (1958), however, successfully reared the Colorado beetle on this plant¹.

¹ WILBRINK (personal communication) reports to have found the Colorado beetle many times on *Solanum carolinense* in the botanical garden of the Michigan State University, and hardly ever on *Solanum rostratum*.

CHEVALIER (1939) found adult Colorado beetles living on and eating from wild plants of *Solanum luteum* MILL. (= *S. villosum* LAMK.) in France, but according to experimental work of BUHR (1956), larvae reared on *S. luteum* died on this substrate, though older larvae (viz. third instars) developed into adults. This is in agreement with JERMY and SÁRINGER (1959) who state that this plant, which is endemic in Hungary, is inappropriate food for young larvae; only for fourth instar larvae and for adults it is said to be suitable.

Solanum nigrum (bitter nightshade) is absolutely refused according to BRUES (1940); TROUVELOT et al. (1933a, b) and BUHR (1956) observed a total mortality on this plant. Though CHIN (1950) states *Solanum nigrum* to be attractive to the Colorado beetle larvae, he does not say that it is possible to raise larvae on this plant.

Though KOZLOVSKY (1936) reared the insect from egg to adult with more or less success on 22 cultivars of *Solanum lycopersicum* (tomato), and FEYTAUD (1936) mentions successful breeding experiments on this plant, BRUES (1940) did not succeed in breeding larvae on this species, nor did TROUVELOT and THENARD (1931). FEYTAUD (1923) observed attack of the Colorado beetle on tomato in the field, as BRUES stated, and the latter mentions that 'quite recent studies in France substantiate this as they have shown that certain varieties of tomato are readily eaten while others are not'. According to JERMY and SÁRINGER (1955b, 1959) development on tomato, as compared with the development on potato, is a little prolonged and the insect shows a considerable mortality. BUHR (1956, 1961) reports that the Colorado beetle displays different behaviour in respect to certain varieties of tomato and to tomato plants of different origin. Results of work of several authors (for an extended survey of literature is referred to BUHR, 1956, 1961) showed that there is a continuous gradation from tomato varieties accepted by beetles and by larvae to those which are absolutely refused. BUHR (1956, 1961) points out that thus far for tomato plants, and for other species as well, the fact was overlooked that the susceptibility of one individual plant to the Colorado beetle attack varied during the different developmental stages of the plant. BALACHOVSKY and MESNIL, however, signalized this phenomenon as early as 1936. Furthermore, according to BUHR (1956), we have to take into account the possibility that genetically homogeneous plant material, grown under different circumstances, may show different susceptibilities.

1.3. HOST PLANT SELECTION

Successful host plant utilization by phytophagous insects unquestionably depends upon properly co-ordinated interaction between the insect and the plant (THORPE et al., 1947; DETHIER, 1947). As has been pointed out by a number of workers, the behaviour that is involved in host plant selection can be divided into several components, of which two stand out immediately (BECK, 1963, 1965; CHIN, 1950; DETHIER, 1953, 1954a; THORSTEINSON, 1953, 1958a, 1960; DE WILDE 1958b; YAMAMOTO, 1957):

1. orientation towards the host plant; in other words: finding the host plant;

2. recognition of the host plant, or host plant acceptance. (Review of literature in DETHIER, 1953, 1954a, 1966 and in THORSTEINSON, 1960).

1.3.1. *Orientation towards the host plant*

When female beetles deposit their eggs on suitable hosts, the hatching larvae do not need to search for acceptable food. When eggs are laid on an unsuitable plant, it is to be expected that in most cases it is beyond the power of the young larvae to seek food elsewhere. Finding the host, therefore, is rather a function of the adult beetles. As in the Colorado beetle a period of feeding precedes the oviposition period, and the heavy gravid female can hardly fly, oviposition normally takes place on the food plant on which the insect matures. In this thesis 'host plant selection' means, unless otherwise denoted, selection of the host plant for the sake of feeding.

The adult beetle may reach the plant by walking or by flight. Usually the distance will be large and that plant odours play a role in host plant finding is a natural supposition. GRISON (1956) states that the smell of cut potato foliage can be perceived by adults at a distance of 4 metres, but JERMY (1958) in an olfactometer did not obtain any response to undamaged potato foliage, contrary to MCINDOO (1926). Upwind orientation towards olfactory stimuli in an air stream, as observed with *Locusta migratoria* (HASKELL et al., 1962) and with *Sitona cylindricollis* (HANS and THORSTEINSON, 1961), effecting the insect apparently at a long distance, was described for *Leptinotarsa decemlineata* by DE WILDE et al. (1969). However, there is no evidence that adult Colorado beetles in the field are attracted to their food plants from a distance. In literature very few data could be found on flight behaviour of the Colorado beetle. Finding the suitable host plant most likely happens accidentally, by trial and error (JERMY, 1958, 1961b; TROUVELOT, 1958; THORSTEINSON, 1960). Foraging is random (not orientated) until the insect comes in the immediate vicinity of the plant. Visual stimuli, positive phototaxis and negative geotaxis are of importance at this stage. Also in larvae optical orientation has been observed (DE WILDE and PET, 1957). Beetles, searching for food (or for sites to deposit their eggs), keep moving in a constant direction guided by a light-compass orientation. This prevents the beetles from roaming at random in a plant stock where host plants are absent, and ensures wandering through a wide space within a short time interval. They climb up every obstacle coming into their visual field and examine it with the contact chemoreceptors; if the obstacle is a plant, and this plant is not adequate for feeding, the beetles leave it, and continue moving in the same direction as before, until they reach a host plant, irrespective of the wind direction (JERMY, 1957, 1958, 1961a, 1961b; JERMY and SÁRINGER, 1959). Although the light-compass orientation increases the probability of host finding, this mechanism works with a very low efficiency. This way of searching for food, with a greater chance for mistakes than for finding a suitable host, will result in a great mortality. Survival of this species is due to the enormous fecundity of the insect (BALACHOVSKY & MESNIL, 1936).

1.3.2. Host plant recognition

While vision, phototaxis, geotaxis, anemotaxis and hygrotaxis play a part in directing the Colorado beetle to the proper environment for oviposition and feeding, the ultimate forces, working at close range and operating in the final recognition of the preferred plant, are largely chemical (DETHIER, 1947; JERMY, 1961a).

RAUCOURT and TROUVELOT (1933, 1936) demonstrated that discs made of elder-pith, which normally is not eaten by the Colorado beetle, will be eaten by the larvae when treated with potato leaf juice. This led to the conclusion that the juice contains sapid constituents, referred to by RAUCOURT and TROUVELOT as 'principes actives', and do not contain feeding inhibitors.

Plants acceptable to the Colorado beetle, such as potato, do not contain, however, phagostimulatory 'odd' substances easily isolated or otherwise available for experimental use (RITTER, 1967). RAUCOURT and TROUVELOT tried to isolate the active matter; *solanine* proved to be inert, as CHAUVIN (1952) and SCHREIBER (1957) confirmed. CHAUVIN partially purified an attractive constituent, containing flavonoid glucosides, which he tried in vain to isolate. Flavons isolated from potato leaves by THORSTEINSON (1960) proved chemotactically inert for Colorado beetle larvae. Because fractions containing sugars and amino acids evoked considerable feeding, he supposed that CHAUVIN's preparation and also the extracts made by RAUCOURT and TROUVELOT contained sugars and amino acids.

HESSE and MEIER (1950) ascribed to acetaldehyde an important role in the food selection of larvae and beetles. Their experiments, however, only indicate that, if acetaldehyde plays any role in the host plant selection of *Leptinotarsa decemlineata*, this role is restricted to host plant finding and has nothing to do with host plant recognition (THORSTEINSON, 1955).

The food plant range of *Leptinotarsa decemlineata* is restricted to some species of the genus *Solanum*. Resistance of the non-susceptible species was traced to the occurrence of rejective constituents of these plants, belonging to the group of the alkaloid-glucosides (BUHR, 1961; BUHR, TOBALL and SCHREIBER, 1958; FRAENKEL, 1959; KUHN and GAUHE, 1947; KUHN and LÖW, 1947, 1948, 1954, 1955, 1957, 1959; KUHN, LÖW and GAUHE, 1950; LANGENBUCH, 1951, 1952; SCHREIBER, 1954a, 1954b, 1956a, 1956b, 1957, 1958, 1963a, 1963b; SCHREIBER et al., 1959; STÜRCKOW, 1959a, 1959b, 1959c; STÜRCKOW and LÖW, 1961; TORKA, 1943, 1949, 1950, 1954, 1958). These studies revealed that, while some of these alkaloids may be toxic, the effect of several others (e.g. *tomatine*) is a sensory inhibition of the feeding activity. The inhibitory influence of *demissine* is somewhat delayed, as was graphically illustrated by DE WILDE (1958b), but here the alkaloid seemed to be relatively less toxic and death could be due to starvation.

The data quoted above led JERMY (1958, 1966) and THORSTEINSON (1960) to the hypotheses that:

- a. oligophagy in *Leptinotarsa decemlineata*, at least, may be determined by 'host avoidance' (WARDLE, 1929) rather than 'host selection,' and
- b. feeding and oviposition are supposed to be highly sensitive to inhibition by

one or more substances, which occur in all plants except in some of the members of the *Solanum* group.

By means of the 'sandwich test' (see page 19) JERMY (1961c, 1966) investigated the palatability of many plant species and he found that, besides the host plants, several others, under which *Pisum sativum* L., failed to show a significant rejective effect. As these plants are much less acceptable than the host plants, it is evident that not only the absence of rejectants and the presence of acceptants, but also a strictly defined amount and combination of the latter is necessary to evoke feeding responses. Under extreme conditions of starvation these 'neutral' plants will be accepted and this explains why TROUVELOT and GRISON (1946) succeeded in rearing larvae of the Colorado beetle on pea leaves (*Pisum sativum*) from hatching up to the second instar.

Persevering in an elaborate research, stimulated by DE WILDE, RITTER (1967) tried to isolate feeding stimulants for the Colorado beetle out of potato leaves. The results obtained indicate the presence of a large number of compounds which Colorado beetle larvae are able to taste. Apparently they are present in just the right concentration to make the potato leaves preferable to other leaves by *Leptinotarsa decemlineata*. Although the existence of 'odd substances' among these components can not be excluded, their role obviously is of minor importance and RITTER concluded that, as far as the Colorado beetle is concerned, a *token feeding stimulant* does not exist. Here it must be taken into account that RITTER offered his substances to the larvae on discs of elder pith; volatile constituents of the plant are left out of consideration. Nevertheless, it seemed evident that *Leptinotarsa decemlineata*'s host plant range is primarily determined by the number and concentration ratio of chemicals to which the chemoreceptors are tuned in a positive and negative way respectively. SCHOONHOVEN and DETHIER (1966) demonstrated electro-physiologically that odour plays an important role in host plant relationship of lepidopterous larvae, and they concluded that food plant discrimination can not be explained solely in terms of acceptance or rejection via maxillary taste receptors, but must also involve the wealth of olfactory information, provided by the antennae and maxillae. Though the work of HSIAO and FRAENKEL indicates that apart from nutrient chemicals (1968a) secondary plant substances also (1968b, c) play a role in the food plant specificity, the research of WARDOJO (1969) with *Leptinotarsa decemlineata* on a meridic diet on which larvae and beetles will feed continuously, proved that, under laboratory conditions, token stimulants are not necessary to evoke feeding behaviour.

1.4. CONDITIONING OF HOST PLANT PREFERENCE

1.4.1. *General*

The question whether or not food plant preference can be modified has been discussed by several authors. For a long time these discussions have been centered around a theory known as 'Hopkins' host selection principle'. According to this theory an insect 'species which breeds in two or more hosts will prefer to

continue to breed in the host to which it has become adapted' (HOPKINS, 1916; HEWITT, 1917). HOPKINS (1917) does not assert this principle to be applicable in general, but according to him it is probably true of many insects. According to CRAIGHEAD (1923), WALSH (1864, 1865) was the first entomologist to state this principle precisely. CRAIGHEAD (1921) found that some species of wood boring beetles behaved according to HOPKINS' principle: in practically all the species experimented with, the adults showed a marked predilection for the host on which they had fed as larvae; continued breeding on a given host intensified the preference for that host. CRAIGHEAD emphasized that the condition of the host has a great influence on host selection: every species prefers an optimum condition of the host.

BRUES (1920), however, rejects a generalization of these findings and believes that these changes in predilection for the host have arisen through mutations and occur only in extremely rare cases and are confined to certain groups.

The work of LARSON (1927) is in agreement with this opinion: adults of *Bruchus quadrimaculatus* FAB. did not show a marked predilection for the host on which they were reared as larvae, and continued breeding on a host did not intensify the preference for that host. Moreover, LARSON stated that adults oviposit as freely on hosts which are unfavourable for larval development as on hosts which are favourable for such development. The host selection principle, as outlined by HOPKINS, does not appear to be valid for *Bruchus*. The objection may be raised that a highly polyphagous insect such as *Bruchus* is not one in which the effect should be expected to manifest itself particularly strongly, and that it would be more reasonable to look for it in an insect with a very limited range of hosts (THORPE and JONES, 1937).

THORPE (1939) has shown that other insects indeed demonstrated the phenomenon of pre-imaginal conditioning. *Drosophila* larvae reared on a food scented with peppermint produced adults which were partial to peppermint odour. This was true even when the last instar larvae were thoroughly washed, proving that influences operative only in the larval stage can modify adult behaviour. In 1956, however, THORPE states that these results have nothing to do with conditioning, but only concern habituation. That larval treatment significantly modifies adult behaviour has also been demonstrated by CUSHING (1941) in oviposition experiments with *Drosophila guttifera*. This induced behaviour, however, is of short duration.

CROMBIE (1944) found that blowflies, first exposed to the odour of menthol, which is normally repellent, in the larval stage became so habituated that the adults tested in an olfactometer were indifferent to the odour. This habituation, however, was only of a few days duration.

Another interesting experiment made by VOLKONSKY (1939, cited by HASKELL et al., 1962) was to offer adult hoppers the choice between the plant they were reared on and another plant known to be palatable; the insect always made their way towards their previous food plant, even if this was nutritionably inferior to the alternative plant.

Experiments by IWAJO and MACHIDA (1961) on host plant preference in the

phytophagous lady beetle *Epilachna pustulosa* KÖNO, which feeds normally on *Cirsium spp.*, and which was forced to feed on potato, showed a positive correlation between the acceptability of potato leaves for parent females and the ability of their larval progeny to develop on this plant. Young beetles preferred potato when they had been reared on it. It is, however, difficult to determine whether this is due to pre-imaginal conditioning to potato or to the high mortality of these insects, possibly resulting in a selection process. When reared directly after hatching for five days on potato, young beetles showed a preference for potato to thistle, while thistle-reared beetles refused potato almost completely. It is not clear, however, what criterion is used to express the preference for potato. Apparently the percentage of beetles eating the foliage of potato is used as such.

Interesting is the observation by SCHOONHOVEN (1967) on the feeding behaviour of the larvae of the tobacco hornworm *Manduca* (= *Protoparce*) *sexta*, which are normally restricted to solanaceous plant species: caterpillars grown on an artificial diet are less selective in this respect and feeding occurs on several plants normally rejected.

Also JERMY et al. (1968) succeeded in inducing preferences for food previously eaten, in *Manduca sexta* and in *Heliothis zea*, but these observations differ from forementioned findings in that no induction was achieved with plants outside the insects host range. A preference once induced is not whiped out by larval moults, the information serving as a basis for the induced feeding habit is probably stored in the central nervous system. However, neither SCHOONHOVEN nor JERMY mention conditioning to foods by larval experience.

According to DETHIER (1947, see also BRUES, 1920) there is no evidence that such conditioning is inherited. All apparent inheritance or strengthening of conditioned preferences can be explained by selection. In our opinion the findings of SLADDEN (see below) are to be classed under selection and eventually under mutation, but not under conditioning. SLADDEN (1934) forced the stick insect (*Carausius* (= *Dixippus*) *morosus* BR.) to feed on ivy (*Hedera helix*), whereas privet (*Ligustrum*) was the food on which the insects were being reared. In the second generation reared on ivy, she found that the individuals more readily accepted this plant than did those of the first generation. In every succeeding generation ivy was more easily accepted than in the former and the insects showed an increased predilection for this plant (SLADDEN, 1935). This increased ability to accept ivy appears to be a function of the time of the year in which the tests were made. Analysis of the results on a monthly basis displays a distinct annual periodicity. Nevertheless, it is difficult to forgo the conclusion that the forced ivy feeding had induced the increased ability to accept ivy in succeeding generations in this parthenogenetic insect (SLADDEN and HEWER, 1938).

Another factor, which has to be taken into account in experiments on host preference, is the variability within an insect species. As IWAO and MACHIDA (1961) demonstrated with *Epilachna pustulosa*: there can be an individual difference between insects collected on the same spot and also a difference between insects of different origin (IWAO, 1959).

As pointed out above, conditioning of larvae to particular foods in some cases may be transmitted through pupation to the adult stage. Oviposition on the preferred foodplant tends to perpetuate the changed preferences.

1.4.2. *Conditioning of host plant preference in Leptinotarsa decemlineata*

MCINDOO (1935) reared *Leptinotarsa decemlineata* on 4 different food plants of the genus *Solanum* viz:

<i>Solanum tuberosum</i>	potato
<i>Solanum dulcamara</i>	bittersweet
<i>Solanum carolinense</i>	horse nettle
<i>Solanum lycopersicum</i>	tomato

In his experiments on plant choice, about 50 beetles were placed in cages of $8\frac{3}{4}$ inches square and $\frac{3}{4}$ inch deep, in which there were 4 leaf discs of one square inch: one of each plant. After 15 minutes the beetles which were in contact with the leaf discs were counted. The total number of 16 replicates was considered adequate as a measure for testing the attraction of the plants. MCINDOO found that all beetles preferred to sit on foodplants on which they had not been reared before, except for females reared on potato, which showed a preference for potato.

Though MCINDOO did not find any pre-imaginal conditioning, LANGENBUCH (1952) states that Colorado beetles collected in the field on potato refused bittersweet (*Solanum dulcamara*), while young beetles in the laboratory reared on bittersweet started to feed immediately on this plant, and they even showed a marked predilection for bittersweet in comparison to potato. This preference was demonstrable during several days.

A change in selectivity was also described by LANGENBUCH (1952): young Colorado beetles during their maturation feeding were less particular in food choice than the old ones.

2. DEFINITIONS AND CRITERIA

Before dealing with the methods, and in view of the questions mentioned on page 2, we will discuss what is here understood by host plant preference and we will define several criteria.

Host plant selection consists essentially of a series of take-it-or-leave-it situations in which the insect either accepts or rejects the plant as food or as oviposition substratum. This does not imply that feeding or oviposition is an all-or-nothing phenomenon. Feeding may continue only through the duration of the initially lowest threshold of response, which is the result of both internal motivation and external stimulation. Should the plant contain a feeding inhibitor or lack feeding stimulants, feeding will continue until the threshold for feeding stimuli rises (THORSTEINSON, 1960); this is graphically depicted by CHIN (1950). There is no need to postulate anything like 'comparison shopping'² to account for our observations on food selection by insects.

Host plant preference, if measured by food consumption of an insect, or a group of insects, in a given choice situation, is the statistical result of a higher frequency of visitation on the one hand and a longer duration of feeding on the other. Host plant selection deals with the external conditions that influence the internal mechanisms that regulate these two phenomena (THORSTEINSON, 1960).

In this thesis feeding preference for a given plant means that when this plant is offered simultaneously with one or more others, the quantity eaten from it exceeds the quantity taken from the others. Oviposition preference for a given plant means that oviposition on this plant outnumbers the oviposition on one or more other plants at the disposal of the insect.

In our opinion MCINDOO's criterion, viz. the number of insects counted on a given food plant (MCINDOO, 1935), is not adequate. More satisfying seemed the food consumption of the insect during a certain time. Initially the amount consumed was calculated by determining the size of the hole in the leaf. However, leaves of different plants differ in thickness, so we turned to weighing to evaluate ingestion by converting the amount consumed into milligrams fresh weight, taking into account the variable thickness of the leaves. The water content too of each leaf will vary from one plant to another and from one moment to the other, but is supposedly not a main factor in food selection, in view of the high general water content of fresh leaves. Therefore we omitted recalculation into fresh weight and of our later experiments food consumption is expressed in milligrams dry weight. These values were obtained by weighing each dried leaf separately.

To guarantee a regular rate of food consumption the insects were starved for 12 to 18 hours before the beginning of the tests, though this will doubtlessly lower the threshold for feeding stimuli and make the insect less discriminative.

² By 'comparison shopping' is meant a deliberate selecting on the part of the insect.

The conditions under which the food is offered to the experimental insects are of great importance. It is necessary that in the test situation:

- a. each beetle (larva) receives equal stimuli from each of the four test plants;
- b. by walking in one direction the beetles (larvae) will stand equal chances to reach any of the discs;
- c. the chance of coming to rest on a given leaf disc by random movement is equal for all beetles (larvae) and for all test plants.

Following MCINDOO (1935) we started our experiments with offering leaf discs of four plant species in each test (see chapter 3.4.1.). By using leaf discs the surface of the leaves presented to the insects is constant and is equal for each plant.

Vertical illumination from above will prevent the beetles from having their motion directed by positive phototaxis.

Temperature should be constant throughout the test and equal in each series of experiments.

Chance factors will be reduced by offering the choice situation repeatedly and in a random schedule.

Mature beetles are very willing to mate, and to exclude the effect of sexual behaviour, experiments should be performed with the sexes separated.

A peculiar phenomenon, also observed by LANGENBUCH (1952), may affect feeding choice experiments: viz. when hungry beetles encounter other beetles already eating from a leaf, the newcomers are probably stimulated to feed on the same spot. This often results in a crowding of beetles, even on resistant plants. The results of such feeding may give the leaves of such plants an appearance comparable with attacked leaves of acceptable plants. In our later experiments, therefore, we tested the beetles individually, to prevent mutual influences.

Conditions under which the experiments are taken should be reproducible. Food plants ought to be in optimal condition and leaves should be in the same physiological stage. From the moment of collecting until the end of the experiment the leaves should be prevented from wilting and kept as turgid as possible. This was achieved by collecting young, still growing leaves in plastic boxes on moist filter paper, providing a saturated atmosphere, and by keeping the discs, and performing the tests in such boxes.

One should bear in mind that experiments on preference for host plants as substratum for oviposition might be influenced by preference for food. Moreover, the food plant will affect the fecundity of the insect and, thereby, influence the oviposition quantitatively.

Fecundity appears an adequate measure for the suitability of a host plant, but not for the food preference of the insect for that plant. It may be that a certain plant is preferred for oviposition though nutritionally inadequate and consequently diminishing fertility. In experiments in which the insect is given the choice of different plants to deposit its eggs on, this effect on fecundity should be known.

In case the insect is free to walk from one of the presented plants to the other, the ratio of eggs laid on both experimental plants provides an effective measure of the oviposition preference for one plant in relation to the other.

3. METHODS AND MATERIALS

3.1. EXPERIMENTAL INSECTS

The beetles were grown after the method described by DE WILDE (1957a) in a greenhouse during the first two years and, after 1961, in a conditioned breeding room, undergoing a 'long day' of 18 hours and a temperature of 25°C, which had proved to be the optimal circumstances for breeding the Colorado beetle as regards growth, fecundity, and preventing diapause (JERMY & SÁRINGER, 1955a; DE WILDE, 1949, 1950, 1953, 1954, 1955, 1957a, 1958a, 1962; DE WILDE & BONGA, 1958; DE WILDE & STEGWEE, 1958; DE WILDE, DUINTJER & MOOK, 1959).

Eggs obtained from the breeding stock of the laboratory were kept in glass boxes of 15 cm diameter and 8 cm high, on a piece of dry filter paper. After hatching the larvae were placed upon the foliage (see below). When growing to later instars the number of individuals was diminished to about 25 larvae per glass box, which proved to be an optimal number. Later on plastic refrigerator boxes were used which were less fragile and more easy to handle.

After 11 up to 18 days, depending on the foodplant used, the larvae were fullgrown and became orange-like instead of reddish; these praepupae were placed in zinc boxes of 22 × 22 × 11 cm³, filled with moistened river sand, in which they dig themselves in immediately. Ten or more days later, the young beetles emerged from the sand; this is the *day of emergence*. The beetles were kept either in glass boxes (or refrigerator boxes) or, when large numbers of beetles were concerned, in cages made of perforated zinc and glass, and covered with a glass plate. Here they were kept for about ten days, feeding on an ample supply of foliage. Beetles thus treated became sexually mature in the intervening time; in this thesis they sometimes are denominated as *mature beetles*.

3.2. PLANTS

Test plant material was originally obtained either from the field or from glass house cultures; but, after 1961, all plants were cultivated in a conditioned greenhouse.

The following plants were grown:

Solanum tuberosum L., potato (perenn.): foliage was originally collected from outdoor plants (cultivar 'Voran'), but in wintertime leaves were taken from plants grown in the greenhouse. In the autumn the cultivars 'Barima' and 'Eigenheimer' were used. Most of the experiments since 1961 were performed with the latter.

Solanum dulcamara L., bittersweet (perennial): initially branches with leaves were cut from shrubs found in the vicinity of the town. After 1961 plants were grown in the greenhouse from seeds collected in the botanical garden of the Agricultural University, Wageningen. Nearly all year round we had growing leaves at our disposal. When these leaves were not as greedily eaten as was ex-

pected from results obtained in 1958 and 1959, three strains were grown from seeds collected in the surroundings of the town. The later experiments were made with a clone derived from cuttings of one bitter-sweet plant.

Solanum carolinense L., **horse nettle** (perennial): this plant was grown because of its role in the food choice experiments of McINDOO (1935) (see page 10). Owing to the pen root of about 5 feet, growing perpendicularly into the soil, and the susceptibility to infestation by mites, we did not succeed in growing large quantities of this herb under greenhouse conditions. Moreover we only obtained sterile seeds and, although it is possible to multiply this plant by cuttings, growing turned out to be too complicated to be a success.

Solanum luteum MILL. (= *S. villosum* LAMK.), **woolly night-shade** (annual): by accident we received seeds of this South European weed in 1958, but later this mistake proved to be a fortunate one. Cultivation is very easy as lots of seeds are produced, which germinate readily.

Solanum nigrum L., **black night-shade** (annual): this plant was grown only once from seeds from the field, as it appeared to be unacceptable to the Colorado beetle.

Solanum (= *Androcera*) *rostratum* DUNAL, **buffalo bur** (annual): this species is supposed to be the original food plant of the Colorado beetle (TOWER, 1906). In a greenhouse this plant is easy to grow, although the seeds need a long time for ripening, and it grows very slowly; it is very susceptible to pests such as mites and aphids.

Solanum lycopersicum L. (= *Lycopersicon esculentum* TRN.), **tomato** (annual): the plants were grown from seeds. On several varieties the insect could not be cultured; for our later experiments 'Wonder van Italië' was chosen because of the high quality of its leaves and the relative low mortality of the larvae bred on it.

One must bear in mind that in breeding plants for insect food only those insecticides can be used which have short action periods and do not leave toxic residues on the foliage. After some unfavourable experiences with spray insecticides 'Kasaerosol' (DDVP) proved to be satisfying.

3.3. BREEDING EXPERIMENTS

3.3.1. *The suitability of the plant as food substratum*

Preceding the choice experiments, the suitability of the testplant as food for the larvae was determined by rearing groups of larvae *ab ovo* on leaves of different test plants, under the same rearing conditions. Duration of larval development and mortality are given in table 1 (page 25).

3.3.2. *Alternate feeding*

As can be seen in table 1 (page 25), *Solanum luteum* is not a suitable food plant. In the beginning of our experiments (DE WILDE et al., 1960) we came to the conclusion that it is toxic. Results of oviposition experiments (see chapter 4.4.4.) raised doubts as regards this toxicity and a comparison was made between

two groups of larvae, one of which was reared *ab ovo* alternately one day on potato leaves and one day on *Solanum luteum*, whilst the other was alternately fed for one day on potato and starved during the next days (see fig. 10, page 26).

3.4. SELECTION OF FOOD

3.4.1. Food choice and food preference

The following method was used to evaluate the preference for food: discs of 2 cm diameter were cut from leaves with a stainless steel tube-punch and placed in large petri dishes of 15 cm diameter. The discs were prevented from wilting by placing them on moistened filter paper. In one petri dish 6 beetles or 6 larvae were placed during 2 hours. In our later experiments plastic refrigerator boxes were used ($19 \times 10,5 \times 7 \text{ cm}^3$) instead of the dishes, and the discs were pinned up with stainless insect pins to prevent them from sticking to the filter paper (see fig. 1), supported by little plastic discs of 0,4 cm diameter. The refrigerator boxes were provided with a 1 cm tempex bottom layer to stick the pins into.

The 6 beetles or larvae were given 12 leaf discs, 3 from each plant species, in a random schedule derived by SLOOFF from BAKKER (1959), in which each beetle or larva was placed between 4 leaf discs as is shown in fig. 31 (page 45). To take into account the effect of the position of the discs, each experiment was composed of 4 replicates of 2 hours (fig. 2, page 16). In this way each possible position was filled once by each plant species. The advantages of this method are clear:

- a. each beetle (larva) receives equal stimuli from each of the four test plants;
- b. by walking in one direction the beetles (larvae) will stand equal chances to reach any of the discs;

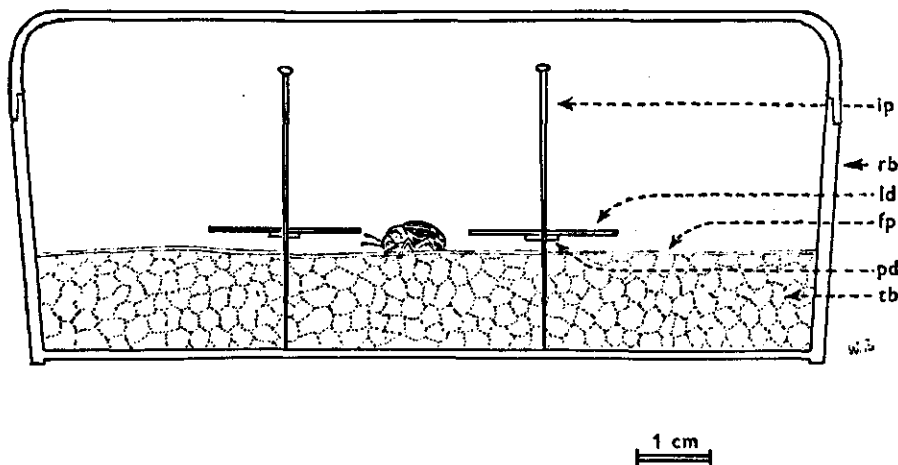
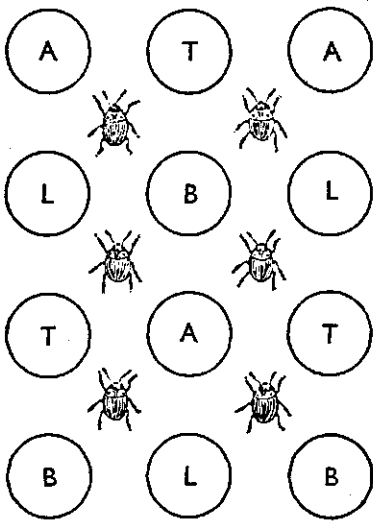
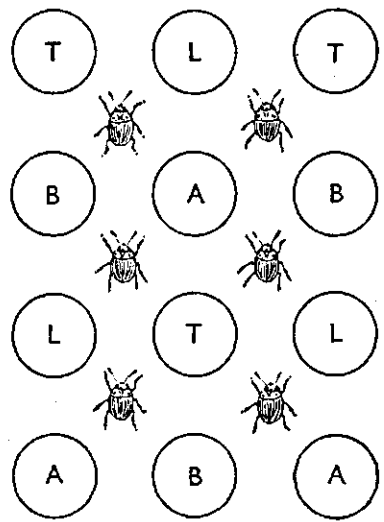


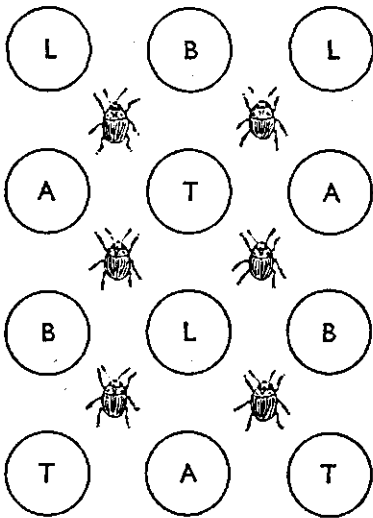
FIG. 1. Food choice test situation according to the scheme given in fig. 6, page 20; ip: insect pin, rb: transparent plastic refrigerator box, ld: leaf disc, fp: moist filter paper, pd: plastic disc, tb: tempex bottomlayer.



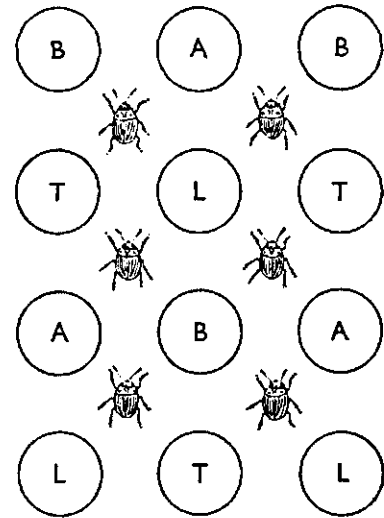
1



2



3



4

FIG. 2. Food choice scheme: 1, 2, 3 and 4: replicates; A: *Solanum tuberosum*, B: *Solanum dulcamara*, L: *Solanum luteum*, T: *Solanum lycopersicum*.

c. the chance of coming to rest on a given leaf disc by random movement is equal for all beetles (larvae) and for all test plants.

The experiments were each repeated 5 to 10 times, and they first took place in a greenhouse in summertime, in which the temperature rose periodically above 30°C due to bright sunshine. Later, after 1961, the experiments were performed in a conditioned breeding room of constant temperature at 25°C or in thermostats.

The petri dishes (refrigerator boxes) were illuminated from above with two 40 Watt TL-tubes; in the thermostats illumination came from aside, with only one TL-tube.

Because we became mainly interested in conditioning *Leptinotarsa decemlineata* to *Solanum dulcamara*, in our later experiments only *S. tuberosum* and *S. dulcamara* were offered (fig. 3). Otherwise the method was unaltered.

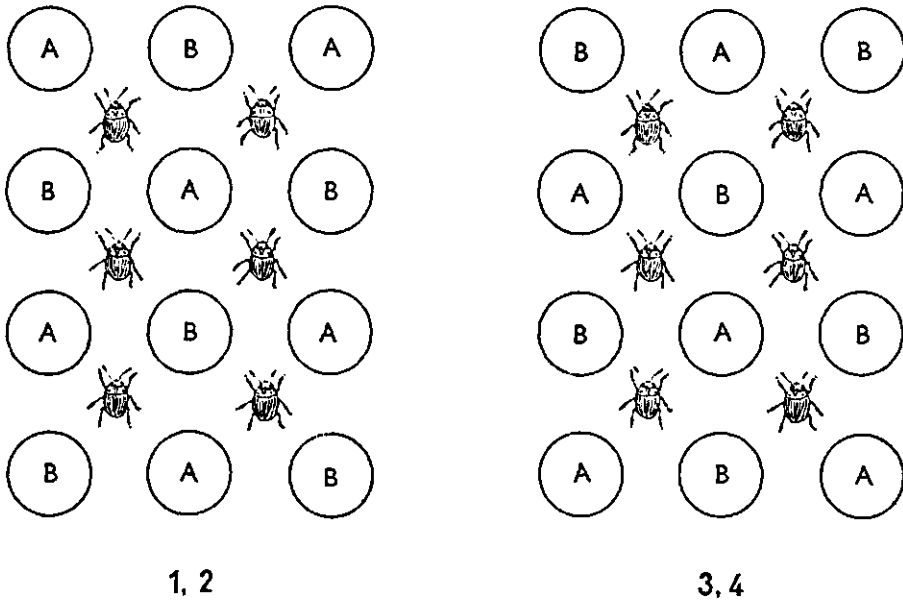


FIG. 3. Food choice scheme: 1, 2, 3 and 4: replicates; A: *Solanum tuberosum*, B: *Solanum dulcamara*.

3.4.2. Evaluation of food consumption

To evaluate the amount of food eaten by the beetles and larvae, the holes in the leaf discs were measured. The discs were placed in a photographic enlargement apparatus and projected on a circle of about 6 cm diameter, drawn on a sheet of paper (see frontispiece). The holes were measured by means of a pole planimeter (after OTT) and recalculated to the original surface dimensions. These data were converted into milligrams fresh weight by multiplying by a factor representing the average weight of one disc (the mean of 50 discs).

To draw the holes in the discs, to measure the surface with a planimeter and

to convert these data into the natural size is very time consuming. The TECHNICAL AND PHYSICAL ENGINEERING RESEARCH SERVICE at Wageningen developed and improved an apparatus after BULGER (1935) and BURGERJON (1962), to measure the holes photo-electrically, which saved much time (fig. 4). The area of the consumed part of the disc could be read directly on the galvanometer scale in percentages of the original surface of the disc.

The holes in the discs were measured by placing the non-consumed part by means of a slide between a light source that illuminated the whole surface of the disc with the same intensity, and a photo cell (ELECTROCELL nr. 781 Selenium 500 micro-Amp. at 1000 lux). The light source, an OSRAM microscope-incandescent

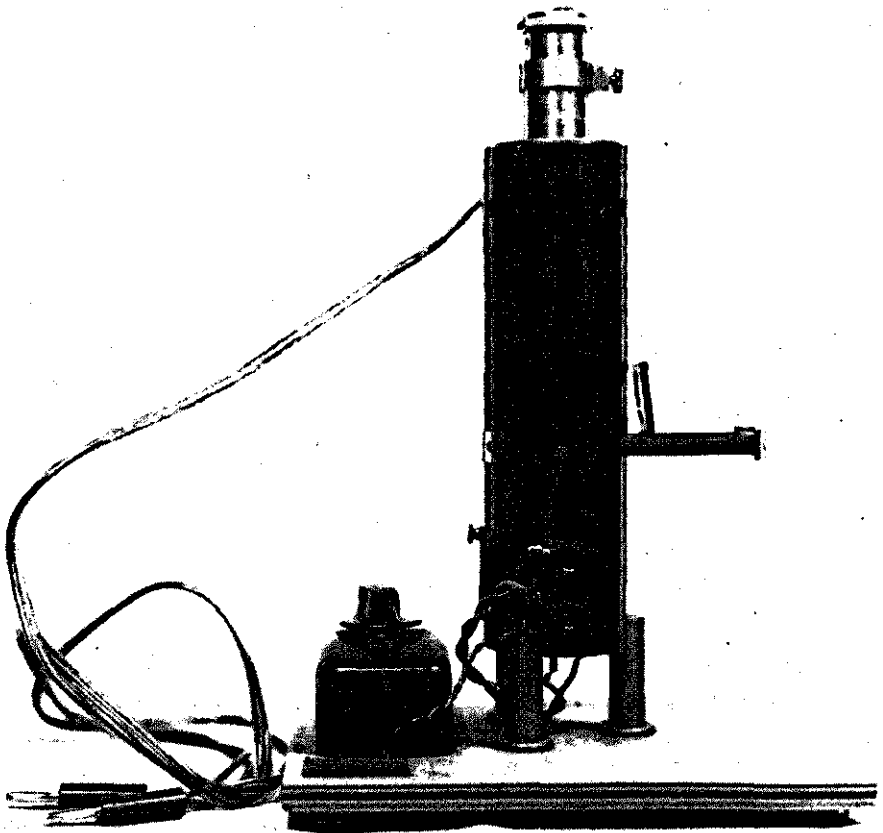


FIG. 4. Apparatus to measure the surface eaten from the leaf discs photo-electrically, developed by the Technical and Physical Engineering Research Service, Wageningen.

lamp (6 Volt, 15 Watt), mounted in a metal tube, was connected with a transformer (2 Volt, 1,5 Amp.); a voltage stabilizer eliminated the mains fluctuations (220 Volt, 50 Hz.). With a potentiometer, connected parallel to the output of the photo cell and the input of the galvanometer (KIPP's model AL 1) the scale deflection of the galvanometer was calibrated at 100% when the slide without a disc was inserted. Thus the consumed part of the disc could be read directly in percentages of the total surface (the galvanometer was used in position 4, which provided a sensibility of $1-4 \cdot 10^{-6}$ Volt/mm).

Not all the leaves of the same plant, however, have the same thickness and the fresh weight varies from one disc to the other. The dry weight of the food consumed is therefore a more reliable criterium. After determination of the percentage of the holes, the discs were dried in a stove of 50°C for 24 hours, and thereupon exposed for 1 hour to the air of the weighing chamber in which they were weighed individually.

If the remaining part of the disc had a dry weight r mg and the percentage of the surface consumed was p %, the dry weight in mg of the part consumed q could be figured out as follows:

$$q = \frac{p}{100-p} r.$$

3.4.3. 'Sandwich'-test

To determine the acceptibility of *Solanum luteum*, the 'sandwich'-test after LANGENBUCH (1952) and JERMY (1961c) was applied. The 'sandwiches' were composed of 2 leaf discs of *S. tuberosum* with a disc of *S. luteum* in between, pinned to one stainless insect pin, and glued together with starch (fig. 5). These were offered simultaneously at 25°C to 5 mature female beetles reared on *S. tuberosum* in 5 separate tests, with control 'sandwiches' consisting of 3 discs of *S. tuberosum*, according to the scheme described on page 20 (fig. 6).

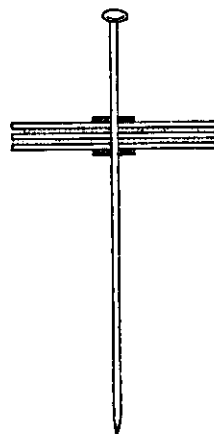


FIG. 5. 'Sandwich' composed of 3 leaf discs, pinned to an insect pin and glued together with starch.

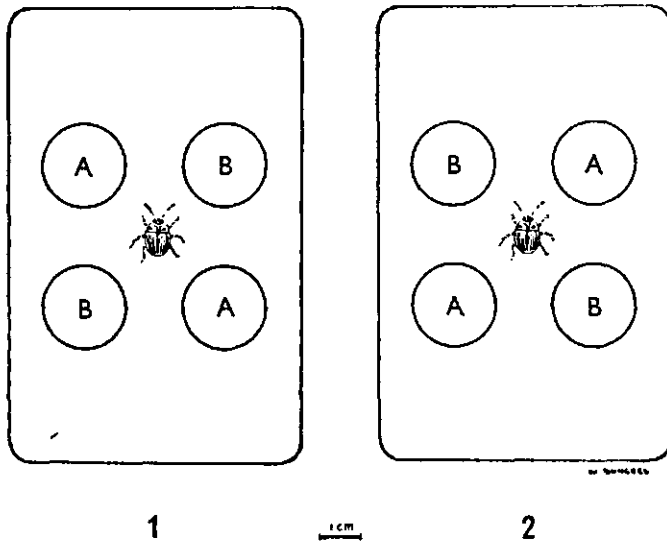


FIG. 6. Food choice scheme: 1, 2: replicates; A: *Solanum tuberosum*, B: *Solanum dulcamara*.

3.4.4. Food choice experiments at different temperatures

Experiments on food choice at different temperatures were carried out in thermostats in which the refrigerator boxes with the discs were brought half an hour before the beetles were released, to achieve the appropriate temperature. To make the results less difficult to interpret, and to avoid mutual disturbance of the beetles, we turned to experimental circumstances under which the beetles were given the choice between two plant species only, and only one beetle was placed in each smaller refrigerator box ($11 \times 7 \times 4,5 \text{ cm}^3$) with four leaf discs. These experiments were repeated up to ten times and were composed of two replicates (fig. 6).

The beetles were kept individually in a glass vial with screw cap at 25°C for about 18 hours. One hour before releasing the beetles in the plastic boxes, the vials were placed in the thermostats to allow the beetles to acclimatize to the particular temperature of the test.

3.4.5. Food choice experiments with impregnated leaves

3.4.5.1. Impregnation with decoction or filtrate

To prevent autolysis of foliage of *S. tuberosum* and of *S. luteum* respectively 100 grams of each were immersed separately during 2 minutes in 300 cc distilled water. After decanting the decoction, the residue was frozen at -15°C for 24 hours, whereupon it was homogenized and filtered in vacuum.

Leaf discs of fresh foliage of *S. tuberosum* were put with one of the four liquids (viz. the decoction and the filtrate of *S. tuberosum* and *S. luteum* respectively) in an erlenmeyer bottle. By a vacuum airpump air was sucked out of the intracellular spaces. By turning off the vacuum pump the spaces filled with the liquid.

At 25°C a series of experiments was set up according to the methods described in fig. 6 in which groups of 5 mature female beetles, reared on *S. tuberosum*, were offered separately the choice between leaf discs of:

1. *S. tub.* and *S. luteum*
2. *S. tub.* and *S. tub.*, impregnated with decoction of *S. tuberosum*
3. *S. tub.* and *S. tub.*, impregnated with decoction of *S. luteum*
4. *S. tub.* and *S. tub.*, impregnated with filtrate of *S. tuberosum*
5. *S. tub.* and *S. tub.*, impregnated with filtrate of *S. luteum*
6. *S. tub.* and *S. tub.*, impregnated with *distilled water*.

3.4.5.2. Impregnation with leaf juice

In the same way as described above leaves of *S. tuberosum* were impregnated with centrifuged juice of fresh foliage of *S. tuberosum* and of *S. luteum*, squeezed out of the leaves with a little hand press and centrifuged to separate the solid particles during 60 minutes and with 30.000 r.p.m.

At 25°C a series of experiments was set up as described above, in which groups of 5 mature female beetles, reared on *S. tuberosum*, were offered separately the choice between leaf discs of:

1. *S. tub.* and *S. luteum*
2. *S. tub.* and *S. tub.*, impregnated with *distilled water*
3. *S. tub.* and *S. tub.*, impregnated with juice of *S. tuberosum*
4. *S. tub.* and *S. tub.*, impregnated with juice of *S. luteum*
5. *S. tub.*, impregnated with juice of *S. tuberosum* and *S. tub.*, impregnated with juice of *S. luteum*.

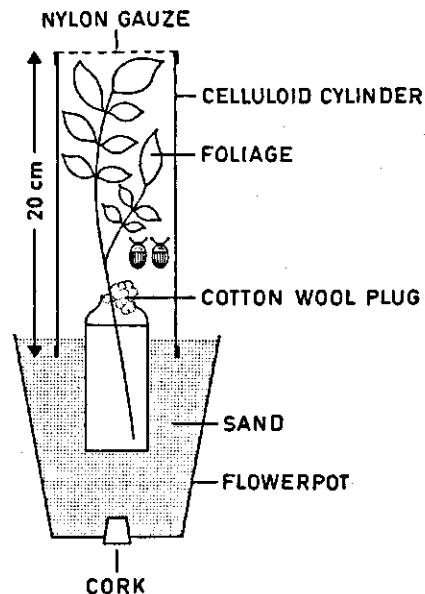


FIG. 7. Arrangement for testing fecundity and oviposition preference.

3.5. OVIPOSITION

3.5.1. *Selection of the host plant for oviposition*

Isolated pairs of newly emerged beetles of the same age were placed on branches of the plant species on which they were reared, in plastic cylinders as shown in fig. 7. Oviposition was recorded daily. After 10 days the males were removed to prevent them from influencing the behaviour of the females. The presence of males is of importance: unfertilized females do not deposit their eggs in masses, but scattered all over the surface.

After a regular rate of oviposition was obtained, the beetles were divided into series, each consisting of 3 sets of 5 to 10 beetles. Within each series one set was given only the plant they were bred on, one set only one of the test plants and one set was given branches of both plants in competition. The aim was to offer the beetles comparable surfaces of the different plant species. The duration of the experiments varied with the purpose of the test, and is clear from the graphs. In those experiments, which were performed in the conditioned room, the prevailing temperature was 25°C; the relative humidity was 60%.

3.5.2. *Oviposition choice at different temperatures*

Oviposition experiments at different temperatures took place in 4 climate chambers in which the temperature, the relative humidity and the day-length could be varied. The temperature was kept at 15°, 20°, 25° and 30°C respectively; the relative humidity at 80% and day length at 18 hours, exactly synchronous with the photoperiod in the breeding room.

Note: in all these experiments the foliage was changed daily in the morning.

3.6. HOST PLANT FINDING

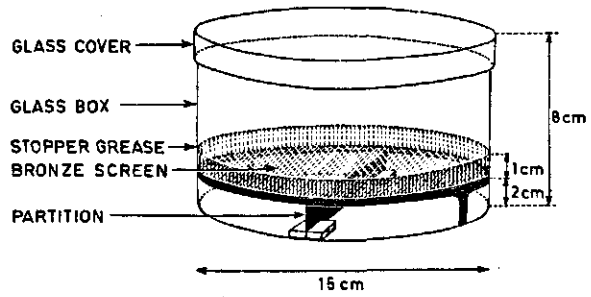
3.6.1. *Screen test*

In a cylindrical glass box, 15 cm in diameter and 8 cm high, (fig. 8), on an iron ring of the same diameter as the inside of the bottom, a bronze screen was soldered. The ring was supported by two legs of 2 cm and by a vertical partition which divided the space underneath in two equal parts. Stopper grease prevented the beetles from climbing the wall.

To eliminate influence of light a sheet of filterpaper around it gave a white wall to the glass box, which was placed in an open zinc canister (height 11 cm; bottom 22 × 22 cm²) over which a lamp (75 Watt) burned, 25 cm above the glass cover. The whole was mounted on a turn table, which was turned every 15 seconds 45° counterclockwise, to exclude possible stimuli from one or more directions. Temperature varied between 22° and 28°C.

In the glass box on the middle of the screen two sexually mature beetles were released after a starvation period of about 2 hours. During 5 minutes just before each turn of the turn table, the distribution of the beetles over the two

FIG. 8. Screen test.



halves of the screen was noted every 15 seconds by counting the numbers of beetles walking on one of the two halves. These numbers were accumulated. When no preference exists for one of the screen halves distribution is recorded as: 20:20.

When one of the halves of the glass box is more attractive than the other (e.g. because foliage is placed underneath the screen), the beetles will dwell there the longer as the attraction is stronger. They will decrease their speeds or even stop for a while and sway their antennae with increased frequency; while in the

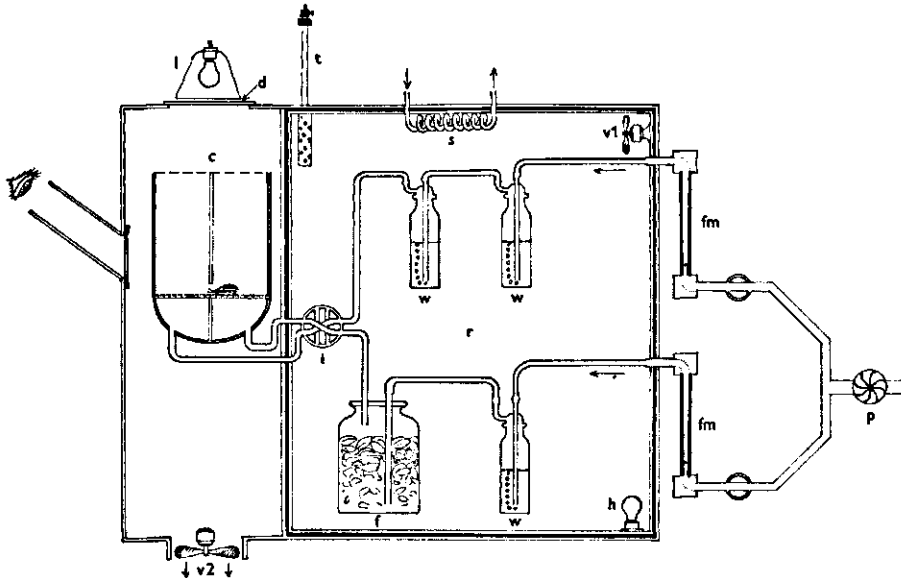


FIG. 9. Olfactometer. c: glass cylinder, d: light diffusion septum, f: 2 liter jar containing cut potato foliage, fm: flowmeters, h: heating lamp, i: cock serving to interchange control air-stream and experimental air-stream, l: light source, p: pump drawing in air filtered by an activated carbon filter, r: conditioned room, s: water spiral for permanent cooling, t: thermostat, connected with h, v₁: electric fan, turbulating the air in r, v₂: electric fan, sucking away the air in c, w: water bottles for moistening air streams.

non-attractive part, they will walk in a steady way. The total number of beetles counted during 5 minutes in one half was considered a measure of attractiveness in *that* part of the glass box. Each experiment was repeated 10 times; the totals were added up and the average is taken as the result (Fig. 42a, page 59).

3.6.2. *Olfactometer test*

The results of the screen tests are compared with those obtained with an air current olfactometer, modified after WIETING and HOSKINS (1939) and LAARMAN (1955) (see fig. 9).

Two currents of air, one of which led through a 2 liter jar containing cut potato foliage, the other serving as control, were led through tubes made of glass and silicon rubber, both controlled by flowmeters, via a perspex hemisphere, through a perforated perspex plate into a glass cylinder. In this way an analogous situation was created to that of the screen test. Stopper grease restrained the beetles from climbing the wall. To prevent turbulence of the two air streams, the cylinder was divided into two halves by a perspex partition, mounted over the perforated bottom in such a way that it allowed the beetles to pass underneath. The air in the cylinder was sucked away by a fan underneath the space in which the cylinder was mounted.

Illumination by a 75 Watt lamp from above through a light diffusion septum appeared to be important. The temperature of the air stream was conditioned at 25°C.

4. EXPERIMENTAL SET UP AND RESULTS

4.1. THE SUITABILITY OF THE PLANT AS A FEEDING SUBSTRATUM

To test the suitability of the plants used, we placed numbers of eggs on the test plants. The numbers of eggs we used varied with the mortality we expected on the ground of experience acquired from former experiments. Experiments were carried out between February and May 1962.

Because nearly all eggs usually hatch, the difference between the number of eggs and the number of praepupae was taken as a measure for 'larval mortality'; the difference between the number of praepupae and the number of emerging beetles as a measure for 'pupal mortality'. Mortality and average duration of larval development were taken as a measure for suitability of the plant as food substratum. As can be seen in table 1, *Solanum nigrum* can not be used as food substratum by the Colorado beetle larvae, nor can *S. luteum*, though a few larvae developed into beetles. Rearing on *S. tuberosum* showed a larval mortality of 12%, which can be considered a normal mortality. *S. dulcamara* induced a mortality three times as high, but still proved to be a suitable plant to breed on. Though mortality on *S. lycopersicum* was more than 60%, still rather large amounts of beetles were produced.

TABLE 1. Developmental duration and mortality

host plant species	number of eggs	number of praepupae	average duration of larval development (days)	larval mortality		pupal mortality	
				number	%	number	%
<i>S. tub.</i>	249	218	18,9	31	12,4	7	3,2
<i>S. dulc.</i>	165	104	18,7	61	36,9	11	10,6
<i>S. lyc.</i>	159	60	18,3	99	62,3	5	8,3
<i>S. lut.</i>	711	24	20,2	689	96,6	6	25,0
<i>S. nigr.</i>	709	4	19,7	705	99,4	3	75,0

The duration of the development did not differ very much for the different food plants.

These results agree very well with those of 1959 (DE WILDE, SLOOFF and BONGERS, 1960). At that time the conclusion was drawn that *S. luteum* was toxic, but that the unsuitability of *S. lycopersicum* apparently was due to its being rejected by the Colorado beetle.

4.2. ALTERNATE FEEDING

Doubts about the toxicity of *S. luteum*, arisen by oviposition experiments (page 50), led us to compare the development of four groups of larvae, *ab ovo* reared respectively on potato (fig. 10a), on *S. luteum* (fig. 10b), on potato, but

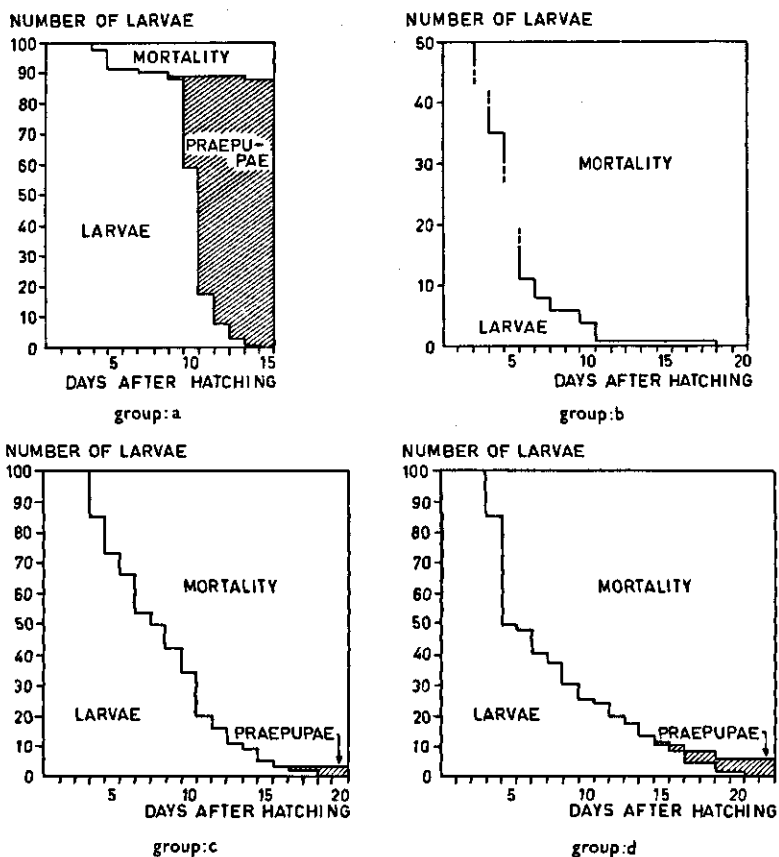


FIG. 10. Alternate feeding. a: (control), development of 100 larvae reared on *Solanum tuberosum*, b: development of 50 larvae reared on *Solanum luteum*, c: development of 100 larvae reared on *Solanum tuberosum* but starved every second day, d: development of 100 larvae reared alternately one day on *Solanum tuberosum* and one day on *Solanum luteum*.

starved every second day (fig. 10c) and one group alternately one day on potato and one day on *S. luteum* (fig. 10d), as described on page 13.

All of the 50 larvae reared on *S. luteum* died before reaching the praepupal stage, while 88 out of 100 larvae reared on *S. tuberosum* became praepupae before the 16th day. Out of 100 larvae, starved every second day, 3 larvae became praepupae, whereas 97 larvae died within 18 days; 5 larvae out of 100, alternated fed on potato and on *S. luteum*, reached the praepupal stage and 95 died, the last on the 21st day.

The graphs fig. 10c and 10d show that the decline of the number of larvae, starved every second day, is almost a straight line and the mortality of the larvae, alternately fed on potato and *S. luteum* is a little retarded compared with the former group. The larvae did feed on *S. luteum*, which apparently lengthened the duration of life. If *S. luteum* were toxic, mortality of the last group should

not be retarded but rather accelerated. The conclusion can be drawn that *S. luteum* is no more toxic than tomato, it only is more rejected by the larvae.

4.3. FOOD CHOICE EXPERIMENTS

4.3.1. Conditioning to the host plant

In preliminary experiments feeding tests were set up in the greenhouse with petri dishes containing discs of leaves of *S. tuberosum* (cultivar 'Vorán') on the one hand and *S. dulcamara* (B), *S. lycopersicum* and *S. luteum* on the other. The experiments were performed with fourth instar larvae, with newly emerged beetles and with 10 days old sexually mature beetles, both males and females. Larvae and beetles were reared on the plants mentioned above. The data on food intake during 8 hours by larvae and adults respectively are summarized in figs. 11 and 12. Due to the high mortality only 6 *luteum*-reared beetles were obtained; representative experiments on food choice could not be made.

All larvae appeared to prefer *S. tuberosum* far above the other experimental plants offered, irrespective of the food plant they had been reared on (fig. 11a). The preference for *S. tuberosum*, therefore, is an innate phenomenon. The same is observed in newly emerged beetles, males as well as females (fig. 11b,c). It follows that the preference for food had not changed during metamorphosis.

In experiments with beetles reared during 10 days on the same plant on which they had been feeding as larvae, those raised on *S. dulcamara* showed a marked preference for this plant, whereas beetles reared on *S. tuberosum* and *S. lycopersicum* preferred *S. tuberosum* (fig. 11d,e).

Beetles reared as larvae on *S. tuberosum*, and during their maturation period on *S. dulcamara* appeared to prefer *S. tuberosum* as did beetles matured on *S. lycopersicum*, whereas beetles matured on *S. luteum* preferred *S. dulcamara* (fig. 12).

4.3.2. Further choice experiments with *Solanum dulcamara*

Because beetles reared as larvae on *S. tuberosum* and during their maturation period on *S. dulcamara* appeared to prefer *S. tuberosum* and not *S. dulcamara*, the conclusion was drawn that a preference for *S. dulcamara* could only be induced (a process we will indicate with the term: 'conditioning') when both larval development and maturation feeding occurred on this plant (DE WILDE, SLOOFF and BONGERS, 1960). Later efforts, however, to reproduce the conditioning of *dulcamara*-reared beetles failed. The results of these experiments are summarized in fig. 13: young beetles, newly emerged, demonstrated a marked preference for *S. tuberosum*, whereas mature beetles did not show any preference but avoided *S. lycopersicum*.

We now expected that these different results were due to one, or several, of the following factors:

1. plant condition
2. plant variety
3. testing conditions

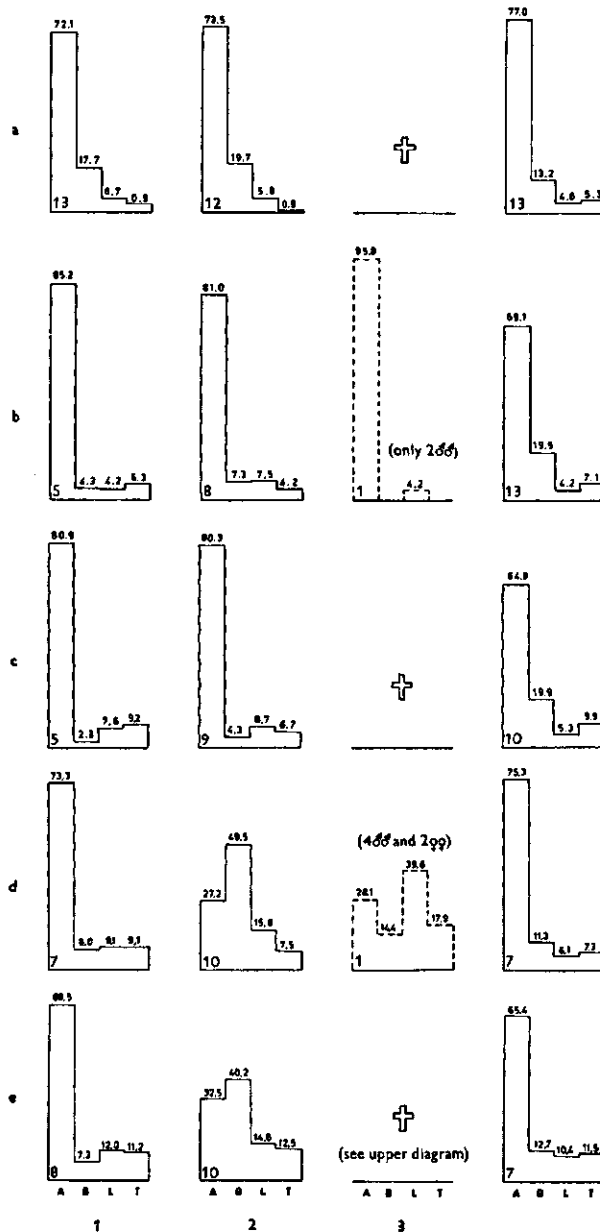


FIG. 11. Percentages fresh weight eaten in food choice experiments at 25 °C by a: fourth instar larvae, b: just emerged male beetles, c: just emerged female beetles, d: mature male beetles, e: mature female beetles; 1: reared on *S. tuberosum*, 2: reared on *S. dulcamara*, 3: reared on *S. luteum*, 4: reared on *S. lycopersicum*; A: consumption from *S. tuberosum*, B: consumption from *S. dulcamara*, L: consumption from *S. luteum*, T: consumption from *S. lycopersicum*. The figures in the diagrams indicate the number of performed experiments. †: all larvae died.

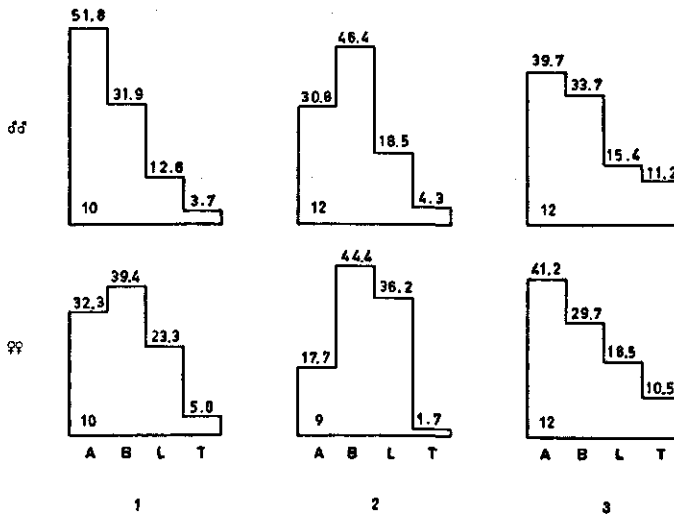


FIG. 12. Percentages fresh weight eaten in food choice experiments at 25°C by mature beetles, being reared as larvae on *Solanum tuberosum* and as adults on 1: *Solanum dulcamara*, 2: *Solanum luteum*, 3: *Solanum lycopersicum*. (For explanation of A, B, L and T: see fig. 11). The figures in the diagrams indicate the number of performed experiments.

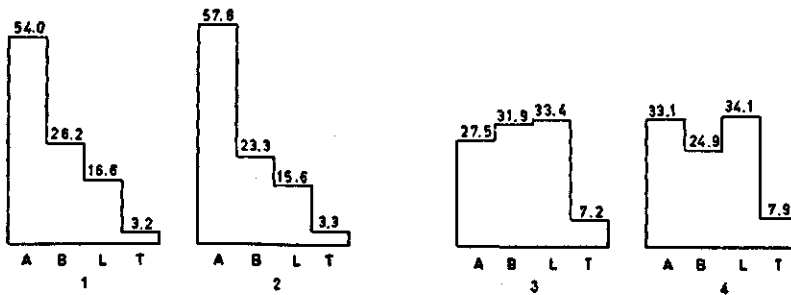


FIG. 13. Percentages fresh weight eaten in food choice experiments at 25°C by mature beetles reared on *Solanum dulcamara* (strain B). (For explanation of A, B, L and T: see fig. 11).

1. newly emerged female beetles (10 experiments);
2. newly emerged male beetles (10 experiments);
3. mature female beetles (10 experiments);
4. mature male beetles (10 experiments).

The plant condition was standardized as much as possible by breeding the experimental plants in a greenhouse, and by selecting more or less similar leaves for the test.

An interesting observation in the experiments on food choice with potato-reared mature beetles was the high relative preference for *S. dulcamara* of mature beetles in cases where this plant had not been eaten during the previous part of their life cycle. It was concluded that the strain of *S. dulcamara* used

might somehow influence the preference. In the experiments in which the conditioning failed, plants have been used grown in a greenhouse, raised from seeds collected in the hortus botanicus 'DE DREIJEN' at Wageningen. Presuming that, apart from the physiological condition of the plants, the strain of the species *Solanum dulcamara* played a role in the results of the experiments, 4 strains were bred in the greenhouse, raised from seeds collected in 4 different localities of the surroundings of Wageningen; one of these (B) originated from the hortus botanicus.

Simultaneously offered to adults, the strain named BA appeared to be most attractive to young beetles as well as to mature beetles (fig. 14). From one of these plants a clone has been bred. In further experiments these plants were used and were offered with *S. tuberosum* cv. 'Eigenheimer' (AE) only, according to the scheme given in fig. 6 (page 20).

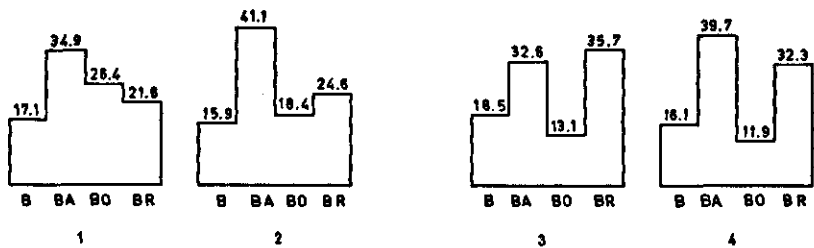


FIG. 14. Percentages fresh weight eaten in food choice experiments at 25°C by beetles reared on *Solanum dulcamara* (strain B), by choice between 4 strains of this plant: B, BA, BO and BR.

1. newly emerged female beetles (5 experiments);
2. newly emerged male beetles (5 experiments);
3. mature female beetles (6 experiments);
4. mature male beetles (4 experiments).

The results of the experiments in which *S. dulcamara* (BA) was offered simultaneously with *S. tuberosum* (AE) to *dulcamara*-reared larvae and imagines are depicted in fig. 15. Fourth instar larvae consumed relatively more of *S. dulcamara* than of *S. tuberosum* as did the mature beetles. The just emerged beetles did not show any preference; differences suggested by the figure are insignificant.

Though we might conclude to a larger food intake from *S. dulcamara* by mature beetles, there is no question of conditioning to this plant as there is no difference in preference when food consumption of mature beetles is compared with that of larvae. Similar experiments with *tuberosum*-reared larvae and young beetles gave the same results (fig. 16); no experiments have been performed with mature beetles.

Although in the preliminary experiments, performed with 120 beetles in 80 replicates, a conditioning to *S. dulcamara* seemed evident, this phenomenon could not be reproduced in our further experiments, even when the most attractive strain of this plant was offered. The strain of plants used plays a major role in the choice for food, but it is clear that the discrepancy between the two series

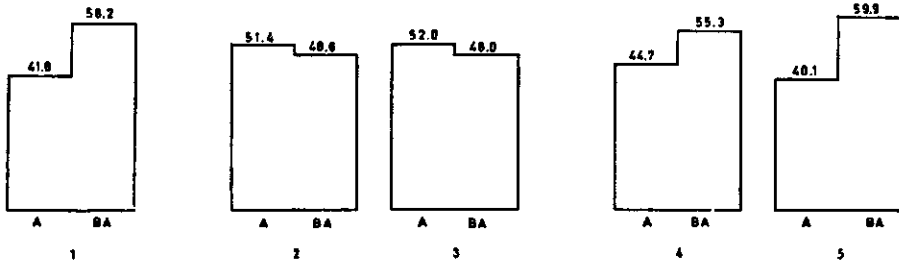


FIG. 15. Percentages fresh weight eaten in food choice experiments at 25°C by larvae and beetles reared on *Solanum dulcamara* (BA). A: consumption from *Solanum tuberosum*, BA: consumption from *Solanum dulcamara* strain BA.

1. fourth instar larvae (10 experiments);
2. newly emerged female beetles (10 experiments);
3. newly emerged male beetles (10 experiments);
4. mature female beetles (6 experiments);
5. mature male beetles (7 experiments).

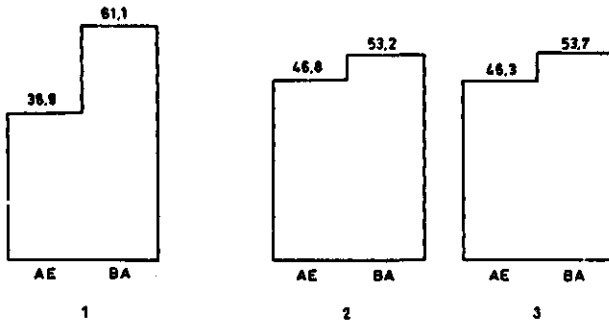


FIG. 16. Percentages fresh weight eaten in food choice experiments at 25°C by larvae and beetles reared on *Solanum tuberosum* cv 'Vorán'. AE: consumption from *Solanum tuberosum* cv 'Eigenheimer', BA: consumption from *Solanum dulcamara* strain BA.

1. fourth instar larvae (6 experiments);
2. newly emerged female beetles (6 experiments);
3. newly emerged male beetles (6 experiments).

of experiments can not be caused only by such differences. Evidently other factors are involved. Although there may be differences in the physiological condition of the plants, it is not likely that these are responsible for the conditioning shown by the initial results. These results were obtained in experiments with to a large degree uncontrolled temperatures. Only the minimum temperature (25°C) could be regulated, and, due to sunshine, temperatures have been recorded up to 35°C and more. Owing to the unambiguous results initially obtained, only little attention has been paid to environmental factors. Now that we could not reproduce conditioning to *S. dulcamara*, it became of great interest to know which role is played by the temperature.

4.3.2.1. Food choice experiments at different temperatures with *Solanum dulcamara*

According to the methods described in chapter 3.4.4. (page 20) a series of experiments on food choice was carried out, performed with female beetles at different temperatures. The figures 17 sqq. show the results: the left graphs show the dry weight eaten from the food plants, and the right graphs represent the percentage dry weight eaten from one of these plants (in most experiments *Solanum tuberosum* AE).

With higher temperatures the rate of food intake increases, but more so with *S. dulcamara* than with *S. tuberosum*. Comparing the amounts of dry weight consumed per hour, we observed that at low temperatures (below about 25°C) *S. tuberosum* is preferred, and at high temperatures (above about 25°C) *S. dul-*

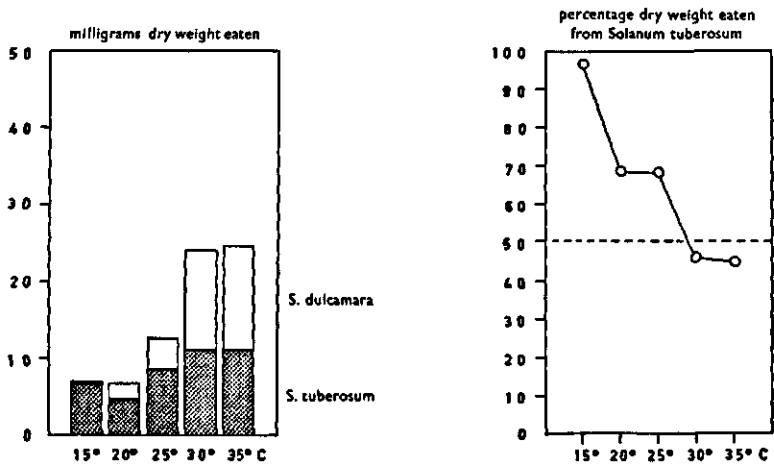


Fig. 17a

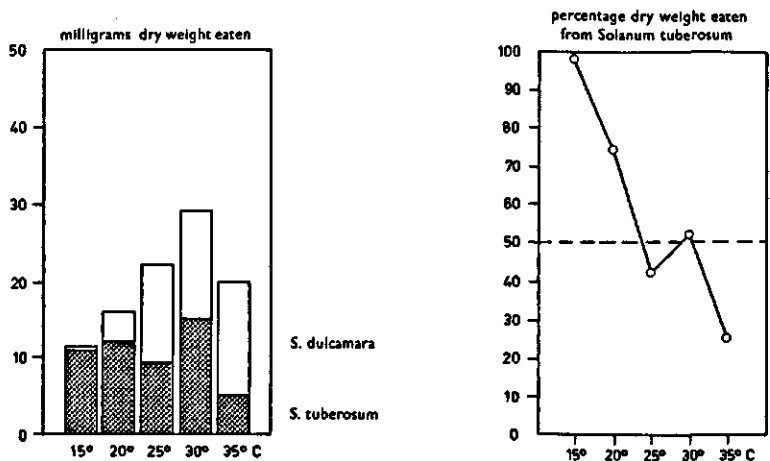


Fig. 17b

camara (figs. 17, 18). This was true for both newly emerged and mature beetles reared previously on either of these food plants. Though in fig. 17c the preference for *S. dulcamara* does not exceed the preference for *S. tuberosum*, this experiment clearly shows that the relative quantities of food taken in are related to the ambient temperature.

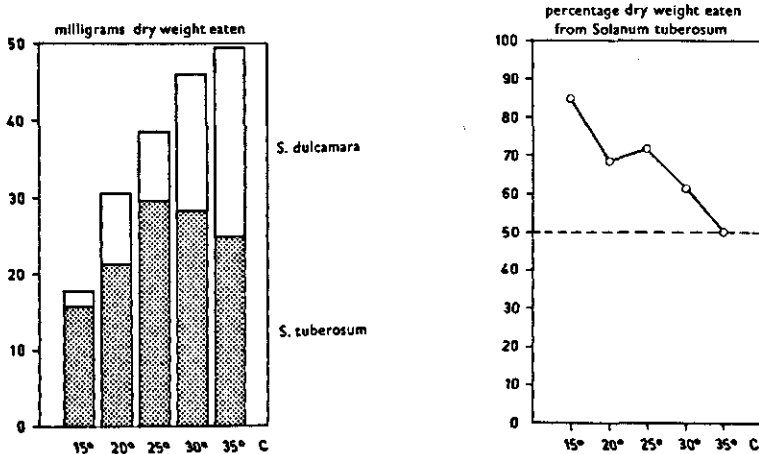


Fig. 17c

FIG. 17. Food choice between *Solanum tuberosum* cv 'Eigenheimer' and *Solanum dulcamara* strain BA during 4 hours (2 replicates) at different temperatures by just emerged (a) and mature (b, c) female beetles reared on *Solanum tuberosum*.

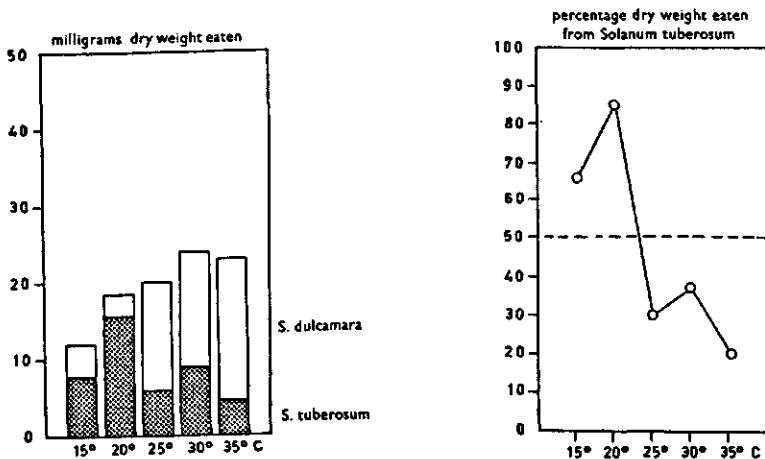


FIG. 18. Food choice between *Solanum tuberosum* cv 'Eigenheimer' and *Solanum dulcamara* strain BA during 4 hours (2 replicates) at different temperatures by just emerged female beetles reared on *Solanum dulcamara* (BA).

4.3.2.2. Conclusion

Surveying the results of the food choice experiments thus far obtained, we conclude that conditioning on *S. dulcamara* does not occur. The food intake depends on the strain and on the physiological condition of the plant. However, neither of them can be responsible for the initial positive results of our conditioning experiments. The ratio of food taken in by mature beetles from *S. tuberosum* and *S. dulcamara* is related to the ambient temperature. With temperatures lower than about 25°C a preference for *S. tuberosum* was observed; with higher temperatures a preference for *S. dulcamara* was apparent.

4.3.2.3. Influence of temperature on the preference for food

The increase of the amount of food intake with the rise of temperature is caused by the enhanced activity of the beetles, which is related to the ambient temperature. Whether or not temperature influences the function of the olfactory or gustatory sense-organs cannot be determined by our type of experiments. On the other hand it is obvious that the plant material will be affected by temperature changes. Although evaporation is suppressed by the ambient humidity in the refrigerator boxes, wilting of the leaf discs could not be altogether avoided. Wilting changes the texture of the discs, the more so in those of *S. dulcamara* than in those of *S. tuberosum*. Leaf-eating insects will prefer turgid leaves to withered leaves. Furthermore, raising the temperature will cause a change in the relative concentration of the compounds of the leaf-substances, e.g: a possibility is that volatile chemicals will be liberated, which affect the chemoreceptors of the beetles. The experiments on food choice mentioned in this thesis do not give a decisive answer to the question how temperature acts on the food plant relationship of the Colorado beetle.

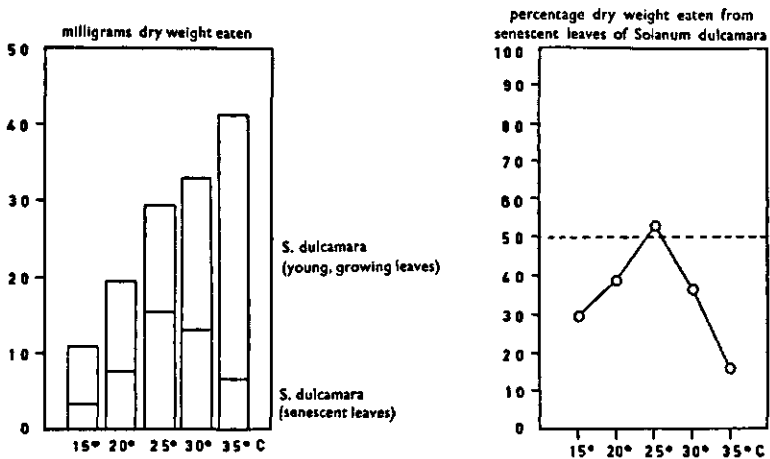


FIG. 19. Food choice between young and senescent leaves of *Solanum dulcamara* strain BA during 4 hours (2 replicates) at different temperatures by female beetles reared on *Solanum dulcamara* (BA).

4.3.2.4. Factors influencing the results

The physiological condition of the plant was apparently of great importance. Mites on *S. dulcamara* cause the beetles to avoid this plant and to eat comparatively more of *S. tuberosum*. Whenever the leaves of *S. tuberosum* are a little senescent, the beetles will prefer young ones of *S. dulcamara*; when, on the contrary, the leaves of *S. dulcamara* are derived from plants which are no longer growing, young leaves of *S. tuberosum* are favoured. Given the choice between plants of the same species (*S. dulcamara*), but of different age, larger quantities of young, growing leaves will be consumed than of the senescent leaves (fig. 19).

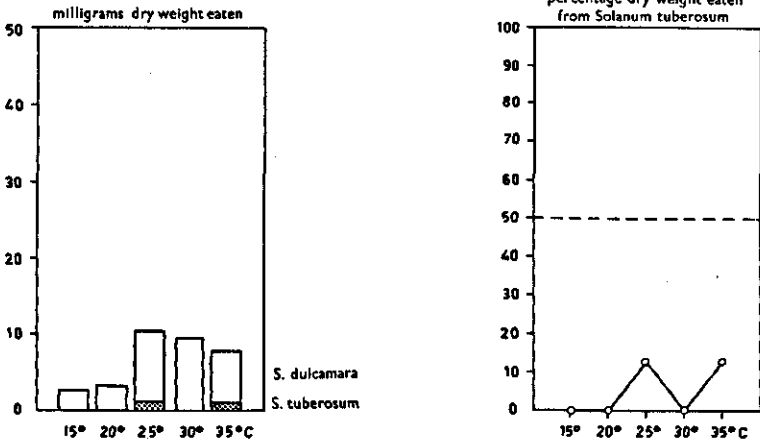


Fig. 20a

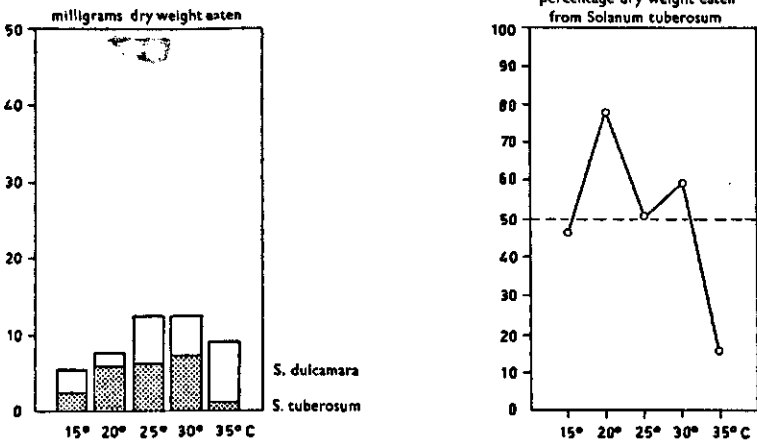


Fig. 20b

FIG. 20. Food choice between *Solanum tuberosum* cv 'Eigenheimer' and *Solanum dulcamara* strain BA during 2 hours at different temperatures by female beetles, which have been bred in and in for 9 generations on *Solanum dulcamara* (a) and on *Solanum tuberosum* (b).

4.3.2.4.1. Inbreeding on *Solanum dulcamara*

Beetles obtained from stocks acquired by inbreeding during several generations on *S. dulcamara* do prefer this plant above *S. tuberosum*, whereas similar beetles reared on *S. tuberosum* prefer this plant.

Comparison of the food consumption of 2 groups of 25 beetles, one group which had been bred in and in for 9 generations on *S. dulcamara* (fig. 20a) and the other derived from the laboratory stock (fig. 20b), which experiments were performed on the same day with the same plant material, also indicates that the first group showed a marked predilection for *S. dulcamara*, whereas the latter do not.

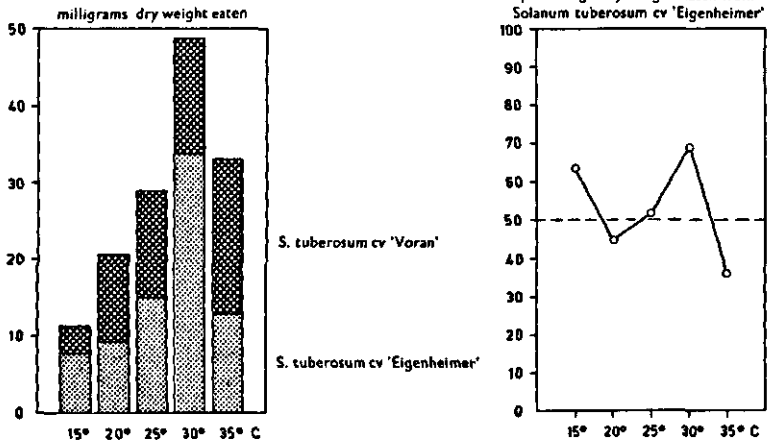


Fig. 21a

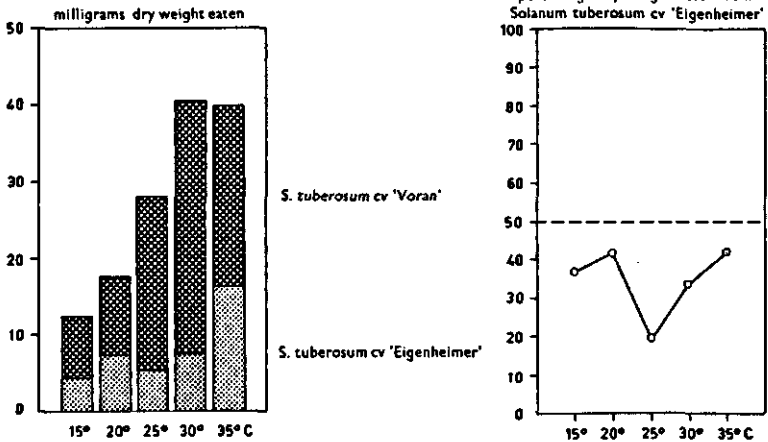


Fig. 21b

FIG. 21. Food choice experiments between 2 cultivars of *Solanum tuberosum*, viz 'Eigenheimer' and 'Vorán' during 4 hours (2 replicates) at different temperatures by mature female beetles reared on *Solanum tuberosum* cv 'Vorán' (a) or on *Solanum dulcamara* strain BA (b).

4.3.2.4.2. Two cultivars of *Solanum tuberosum*

One might object that in the experiments which showed 'conditioning' to *S. tuberosum* the cv 'Voran' was given, while in later experiments the cv 'Eigenheimer' was offered. It is not probable that replacing the former by the latter makes any difference, because when, at different temperatures, both varieties were offered simultaneously to beetles reared on *S. dulcamara* or on *S. tuberosum* ('Eigenheimer'), no marked preference for one of the two was observed (fig. 21).

4.3.3. Choice experiments with *Solanum carolinense*

Following the experiments of McINDOO (1935), in the experimental set up, described in chapter 3.4.1. (fig. 2) *S. luteum* was replaced by *S. carolinense*. The results of feeding tests with insects reared on *S. carolinense* at 25°C are given in fig. 22. All beetles, immature as well as mature, consumed more from *S. tuberosum* than from all other plants together. Again, no conditioning was observed.

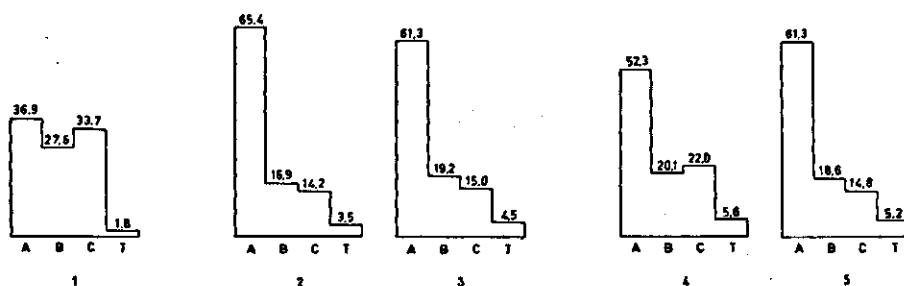


FIG. 22. Percentages fresh weight eaten in food choice experiments at 25°C by larvae and beetles reared on *Solanum carolinense*; A: consumption from *Solanum tuberosum*, B: consumption from *Solanum dulcamara*, C: consumption from *Solanum carolinense*, T: consumption from *Solanum lycopersicum*.

1. fourth instar larvae (10 experiments);
2. newly emerged female beetles (10 experiments);
3. newly emerged male beetles (10 experiments);
4. mature female beetles (6 experiments);
5. mature male beetles (2 experiments).

4.3.4. Choice experiments with *Solanum rostratum*

Results of experiments at 25°C with insects reared on *S. rostratum*, according to the methods described in chapter 3.4.1. (fig. 2) in which *S. luteum* was replaced by *S. rostratum* (fig. 23), indicated a rather marked preference for this plant by newly emerged beetles. In mature beetles this preference was less obvious, but, at that time, the physiological condition of the plants was disturbing the results. Notwithstanding this, the results of experiments at different temperatures (fig. 24a) indicate that for this species too the ratio of food intake is related to the ambient temperature. Whether or not *S. rostratum* in good condition will be favoured at higher temperatures, contrary to the preference at lower temperatures is not certain, but seems likely.

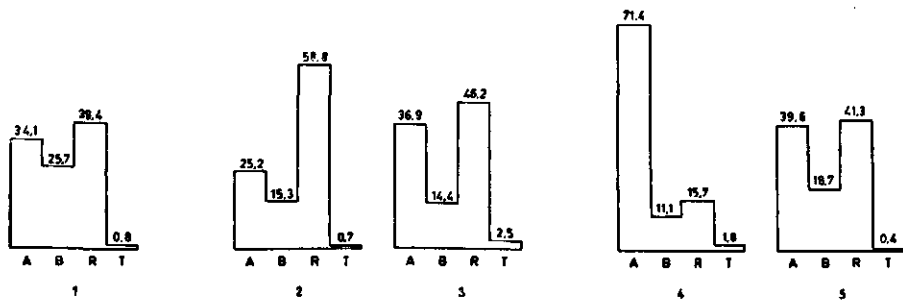


FIG. 23. Percentages fresh weight eaten in food choice experiments at 25°C by larvae and beetles reared on *Solanum rostratum*. A: consumption from *Solanum tuberosum*, B: consumption from *Solanum dulcamara*, R: consumption from *Solanum rostratum*, T: consumption from *Solanum lycopersicum*.
 1. fourth instar larvae (10 experiments);
 2. newly emerged female beetles (3 experiments);
 3. newly emerged male beetles (4 experiments);
 4. mature female beetles (2 experiments);
 5. mature male beetles (4 experiments).

A comparison of fig. 24a with fig. 24b suggests an enhanced preference for *S. rostratum* by beetles reared on this species.

4.3.5. Choice experiments with *Solanum lycopersicum*

Due to the apparent rejective properties of *S. lycopersicum* no conditioning on this plant could be obtained in the preliminary experiments (fig. 11). Experiments with different temperatures point out that the temperature does not have any influence on the ratio of food intake when *S. tuberosum* and *S. lycopersicum* are offered simultaneously (fig. 25a). It should be noted that maxillectomy did not alter the preference either (fig. 25b) (cf. WALDBAUER and FRAENKEL, 1961; WALDBAUER, 1962, 1963).

4.3.6. Choice experiments with *Solanum luteum*

In all experiments *S. luteum* is rejected as food by *Leptinotarsa*. The results with food choice experiments at different temperatures (fig. 26) demonstrate that the rejection of *S. luteum* does not depend on the temperature.

4.3.6.1. The 'toxicity' of *Solanum luteum*

Nearly all larvae offered *S. luteum* for food die within several days; breeding on this plant results in an almost complete mortality (see table 1, page 25). However, beetles reared as larvae on *S. tuberosum*, and given *S. luteum* when adult will die only after a rather long interval. Occasionally a few beetles, reared *ab ovo* on *S. luteum*, could be obtained. These phenomena suggest that this plant species is not toxic, but is only rejected by deterrent properties of the plant. Another explanation could be that the plant is neither toxic nor rejective: a lack of adequate feeding stimulants could prevent the insects from eating this species.

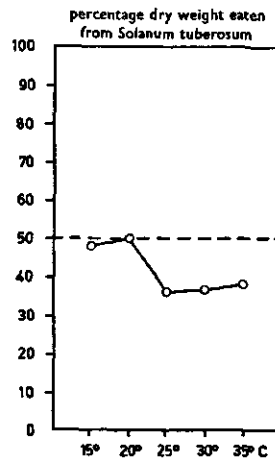
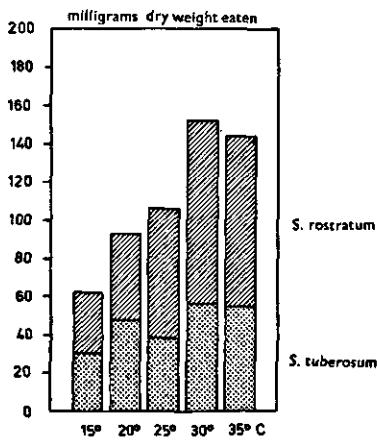


Fig. 24a

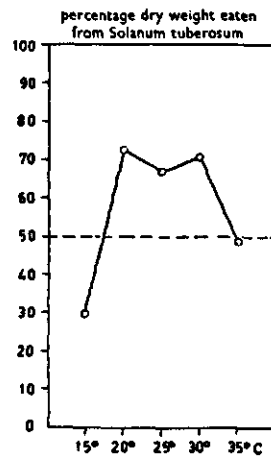
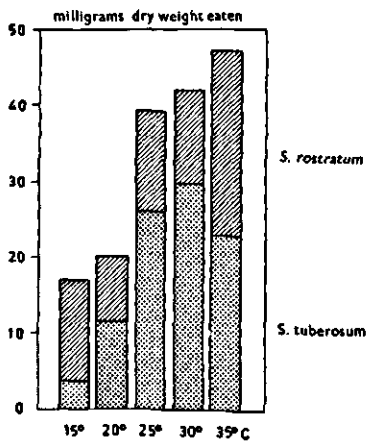


Fig. 24b

FIG. 24. Food choice between *Solanum tuberosum* cv 'Eigenheimer' and *Solanum rostratum* at different temperatures by mature female beetles reared on *Solanum rostratum* (a: the mean of 5 experiments of 4 hours (2 replicates) each) or on *Solanum tuberosum* (b: during 4 hours (2 replicates)).

4.3.6.1.1. Food choice experiments with impregnated leaves

The results of the experiments described in chapter 3.4.5.1., in which groups of five mature female beetles were offered, each beetle in a separate box, the choice between leaf discs of *S. tuberosum* at the one hand and leaf discs of *S. tuberosum* impregnated with decoction or filtrate of *S. tuberosum* or of *S. luteum* on the other, are depicted in fig. 27; the results of similar experiments with centrifuged leaf juice in fig. 28 (page 42).

Again a preference for *S. tuberosum* in relation to *S. luteum* was observed. Impregnation with water or with any liquid obtained from *S. tuberosum* en-

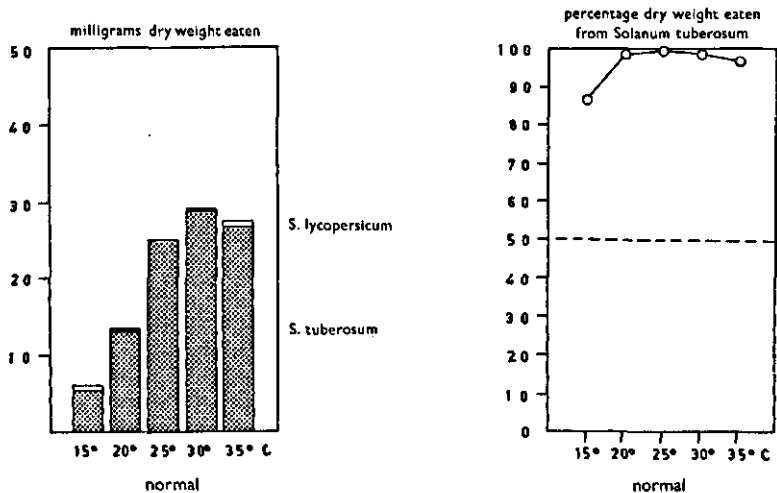


Fig. 25a

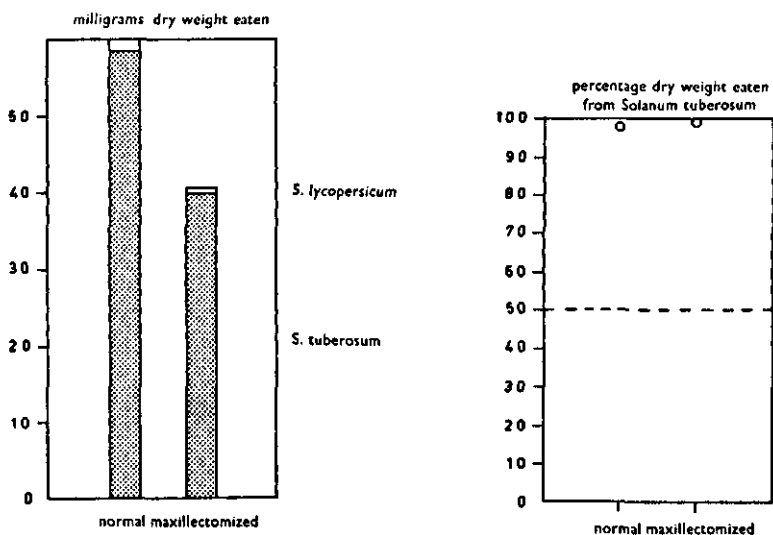


Fig. 25b

FIG. 25. Food choice between *Solanum tuberosum* cv 'Eigenheimer' and *Solanum lycopersicum* cv 'Wonder van Italië' at different temperatures by mature female beetles reared on *Solanum lycopersicum*; a: during 4 hours (2 replicates) by normal beetles, b: during 2 hours by a group of 10 maxillectomized beetles compared with a group of 10 normal beetles.

hanced, or at least did not diminish the relative attractiveness of the leaf discs. Impregnation with derivatives from *S. luteum* diminished the consumption from these discs, though more was eaten from these discs than from leaf discs of *S. luteum*.

These results suggest that *S. luteum* indeed possesses rejecting properties.

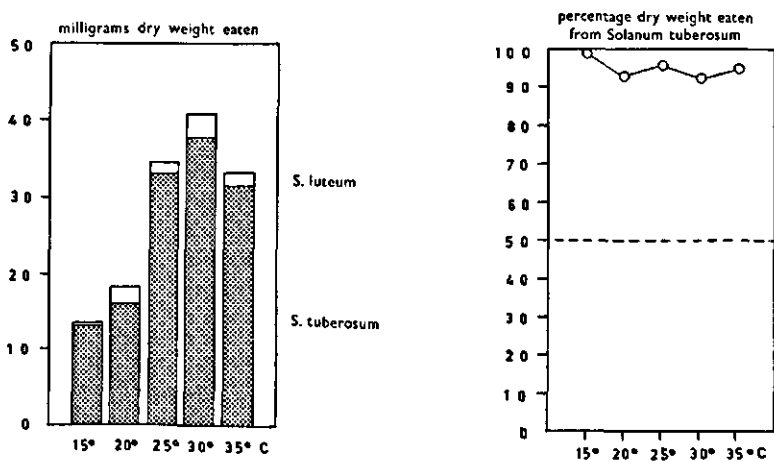


FIG. 26. Food choice between *Solanum tuberosum* cv 'Eigenheimer' and *Solanum luteum* during 4 hours (2 replicates) at different temperatures by mature female beetles reared on *Solanum tuberosum*.

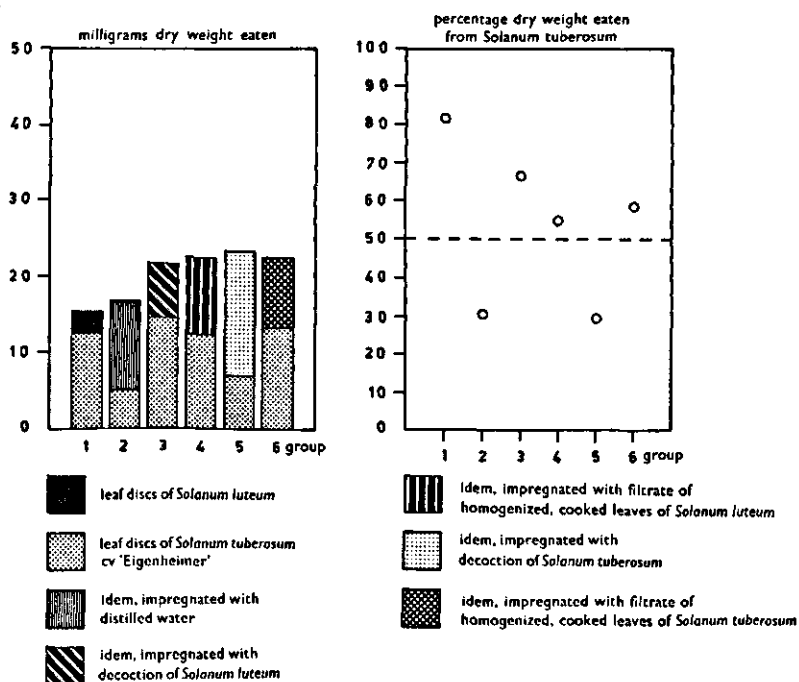


FIG. 27. Food choice during 2 hours at 25°C by 6 groups of 5 mature female beetles reared on *Solanum tuberosum*, in experiments with leaf discs of *Solanum tuberosum* cv 'Eigenheimer' impregnated with extracts derived from *Solanum luteum* and from *Solanum tuberosum* cv 'Eigenheimer'.

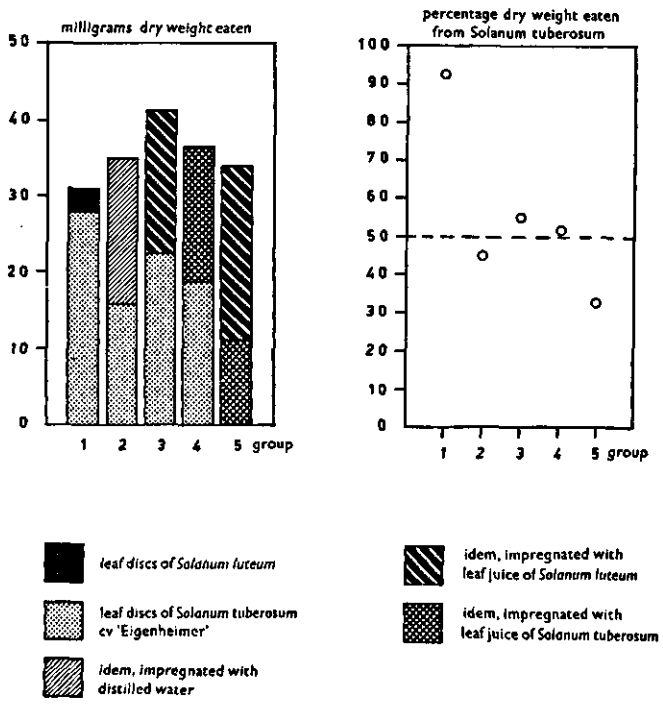


FIG. 28. Food choice during 2 hours at 25 °C by 5 groups of 8 mature female beetles reared on *Solanum tuberosum*, in experiments with leaf discs of *Solanum tuberosum* cv 'Eigenheimer' impregnated with leaf juice from *Solanum luteum* or from *Solanum tuberosum* cv 'Eigenheimer'.

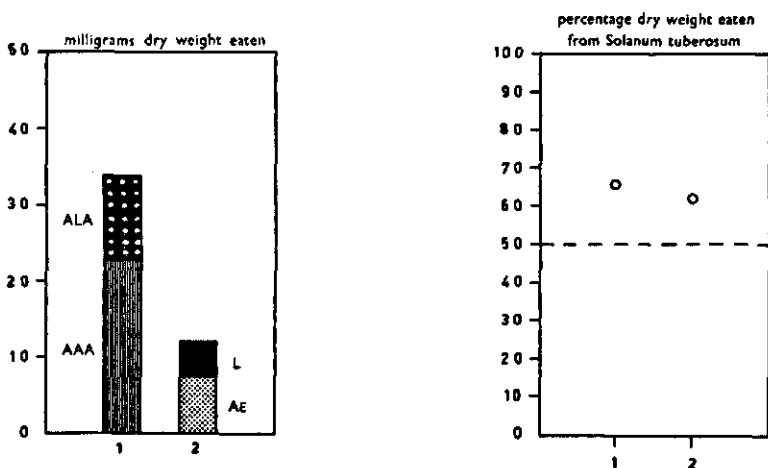


FIG. 29. Food choice during 2 hours at 25 °C between 'sandwiches' composed of 3 leaf discs by 2 groups of 5 beetles reared on *Solanum tuberosum* cv 'Barima'. 1: consumption from the 'sandwich' as a whole, AAA: 'sandwich' with *Solanum tuberosum* cv 'Eigenheimer' between, ALA: 'sandwich with *Solanum luteum* between. 2: consumption from middle disc of the 'sandwich', AE: *Solanum tuberosum* cv 'Eigenheimer', L: *Solanum luteum*.

4.3.6.1.2. 'Sandwich'-test

Of these food choice tests, of which the experimental set up is described in chapter 3.4.3., the results are summarized in fig. 29. The consumption from control 'sandwiches' surpassed the consumption from those containing *S. luteum*. Here again a rejective effect of *S. luteum* is apparent.

4.3.7. The experimental arrangement

4.3.7.1. Observations of the food intake at different temperatures during 24 hours

It is of interest to know whether the time of the day during which the choice experiments are performed, is of influence on the results. In other words: how is feeding activity distributed over the day?

Four groups of 5 mature female beetles, reared in the breeding room (dark period 10 p.m. — 7 a.m.) were kept for 18 hours in climate rooms at 20°, 25°, 30° and 35°C (dark period 8 p.m. — 4 a.m.) on foliage of *S. tuberosum*. Subsequently each beetle was given 4 leaf discs of *S. tuberosum*, according to the experimental set up described in chapter 3.4.4. (fig. 1). During 24 hours the leaf discs were removed and replaced by freshly cut discs every 2 hours. The food intake per two hours, added up of each group is depicted in fig. 30.

Food intake took place during the whole light period and was decreased at night. Also DE WILDE et al. (1959) described a sudden decrease of the food consumption after the beginning of the dark period. The decline in our experiment,

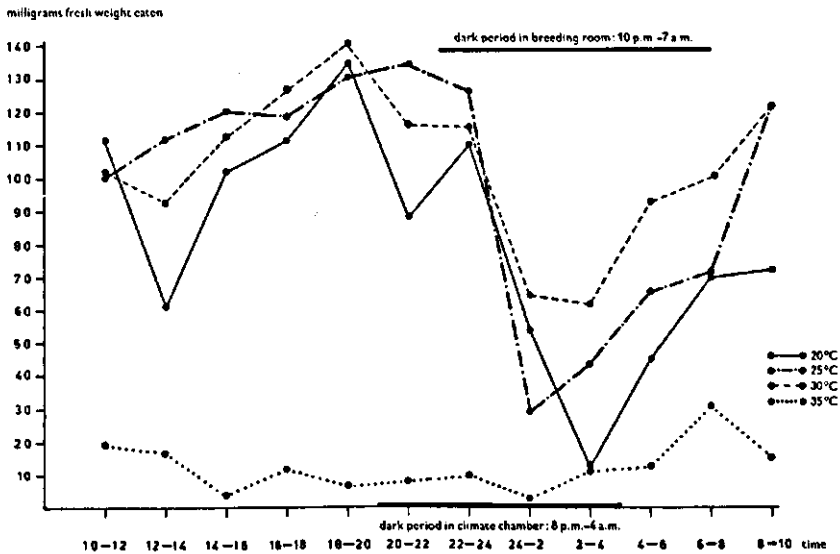


FIG. 30. Food consumption from *Solanum tuberosum* by 4 groups of 5 mature female beetles during 24 hours at different temperatures.

however, coincided with the beginning of the dark period of the breeding room and not with the beginning of the skotophase in the climate chamber. It would seem, therefore, that a feeding rhythm was induced during the pre-treatment and persisted during the time of the experiment.

We may conclude that:

1. experiments on food choice must be carried out during the light period;
2. neither the phase nor the duration of the light period should be changed during a feeding test;
3. the exact hour in the light period during which the test is performed is not relevant.

4.3.7.2. Observations on the behaviour during the food choice experiments

It is of importance to know whether the choice situation indeed gives the beetles the opportunity to make a selection out of the plant species offered. In other words: how do beetles behave during the food choice experiment?

Six female beetles were given the choice between 12 discs of 4 different *Solanum* species (viz: *S. tuberosum*, *S. dulcamara*, *S. luteum* and *S. lycopersicum*) using the experimental set up described in chapter 3.4.1. (fig. 31-1). In fig. 31-2 the track of one of these beetles is drawn and the time interval is referred to by encircled numbers. The part of the leaf disc consumed by this beetle is shaded.

Observing the beetle's activities, we conclude that all discs have been examined. As regards food consumption, we may conclude that the number of times the beetle came across a leaf disc was not related to the quantity of food consumed (table 2). Of course there is a relation between the duration of the sojourn on each disc and the amount of food eaten from it.

Table 2. The behaviour of one beetle in a food choice test

Plant species	Number of encounters of the leaf discs	Minutes duration of the sojourn on each plant species	Estimated part consumed of each plant species
<i>Solanum tuberosum</i>	8	75	1½ disc
<i>Solanum dulcamara</i>	6	15	¼
<i>Solanum luteum</i>	12	< 5	0
<i>Solanum lycopersicum</i>	11	< 5	0

When hungry beetles encounter other beetles already eating from a disc, the newcomers are probably stimulated to feed on the same spot. This sometimes results in a crowding of beetles, even on discs of *S. luteum* or *S. lycopersicum*, which gives these discs an appearance comparable with discs of *S. tuberosum* or of *S. dulcamara*. Testing the beetles individually, therefore, is to be preferred to avoid mutual influences.

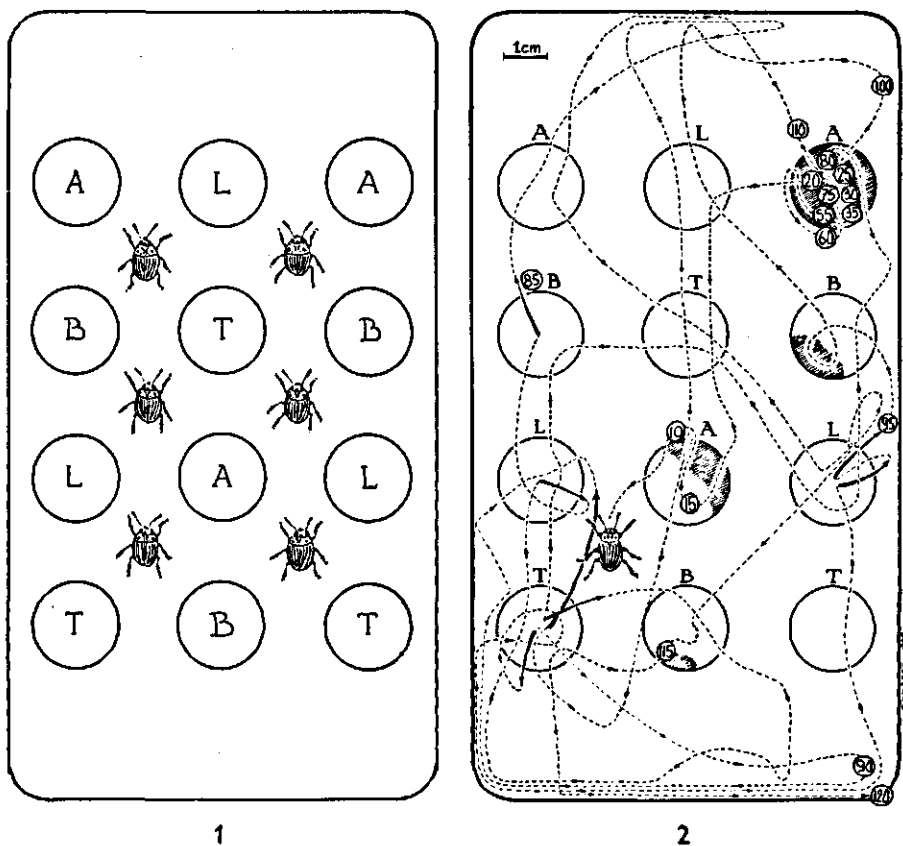


FIG. 31. Food choice test situation. 1: six Colorado beetles are given the choice between 12 discs from 4 different *Solanum*-species, pinned up with stainless insect pins (see fig. 1, page 15); 2: the track of one of the beetles is drawn and the time interval is referred to by encircled numbers; the arrows indicate the places where the beetles climbed up the pins and subsequently tumbled down; the part of the leaf disc eaten by this beetle is shaded.

A: *Solanum tuberosum*, B: *Solanum dulcamara*, L: *Solanum luteum*, T: *Solanum lycopersicum*.

4.3.8. Summary of the results of the experiments on food preference

The results of the preliminary experiments suggest a conditioning of the preference for *S. dulcamara* by breeding the insects during one generation *ab ovo* in their larval and adult stages on this species, whereas control beetles, reared on *S. tuberosum*, do prefer the latter. It must be emphasized that in later experiments, performed under better controlled conditions, these results could not be reproduced.

When *S. tuberosum* and *S. dulcamara* are offered simultaneously, the ratio of food intake, which is in favour of the first at temperatures below about 25°C, is inverted when temperature increases to reach values above 25°C. This, how-

ever, is only valid when plants of optimal condition are compared. Senescent leaves and leaves attacked by mite pests are less attractive than young, growing leaves.

Although differences in attractiveness were observed between different strains of *S. dulcamara*, it is not likely that the results of the preliminary experiments are influenced by the strain of this species used. Nor did different cultivars of *S. tuberosum*: the cv 'Eigenheimer' and the cv 'Vorán' do show the same attraction.

Notwithstanding rearing the insects on *S. dulcamara* does not induce a preference for this species in one generation, inbreeding on *S. dulcamara* during more generations causes a predilection for this plant species. The same seems probable for inbreeding on *S. rostratum*. The preference for this species, when offered simultaneously with *S. tuberosum*, also seems to depend on the ambient temperature.

Neither the consumption from *S. lycopersicum* nor that from *S. luteum* is influenced by temperature.

As also suggested by the oviposition experiments, the food choice experiments too pointed out that *S. luteum* is not toxic. This species apparently is rejective to *Leptinotarsa decemlineata* as a food plant.

4.4. OVIPOSITION

4.4.1. Preliminary experiments

With regard to oviposition host plant choice experiments were performed with *S. tuberosum* (cv 'Vorán', bred in the field) on the one hand and *S. dulcamara* (B), *S. lycopersicum* and *S. luteum* on the other.

Experiments were performed in a greenhouse with three series, each consisting of three groups of seven *tuberosum*-reared female beetles. To check nor-

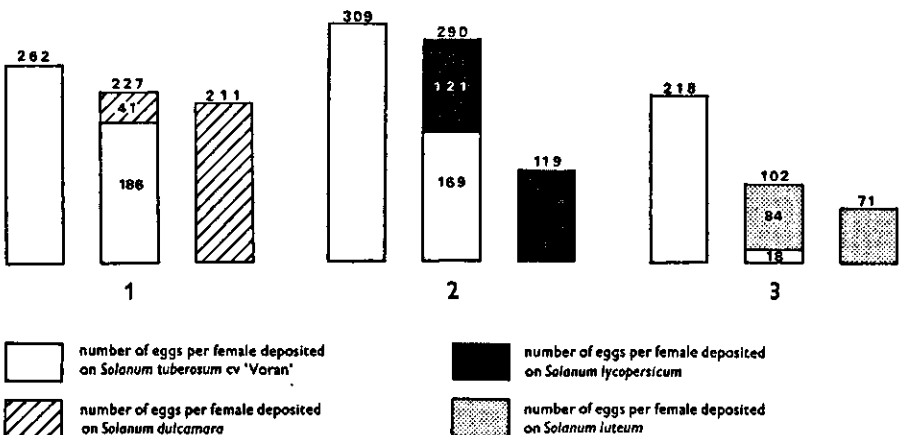


FIG. 32. Oviposition during 5 days on different food plants by 3 series of beetles reared on *Solanum tuberosum* cv 'Vorán'.

mal fertility, of each series one group was given *S. tuberosum* only. To trace the influence of the experimental plant on fecundity, one group of each series was given either *S. dulcamara*, *S. lycopersicum* or *S. luteum*. The third group of each series was given both *S. tuberosum* and *S. dulcamara*, *S. lycopersicum* and *S. luteum* respectively.

The results are given in fig. 32, showing the total number of eggs of each group during 5 days of observation. Fecundity is slightly depressed by *S. dulcamara*, more so by *S. lycopersicum* and heavily depressed by *S. luteum*. *S. tuberosum* is preferred as a substratum for oviposition to *S. dulcamara* and *S. lycopersicum*, but *S. luteum* is favoured more than *S. tuberosum* although *S. luteum* suppresses the fecundity and it is an avoided food plant (see chapter 4.3.).

4.4.2. Further choice experiments with *Solanum dulcamara*

In earlier experiments on food choice between *S. tuberosum* and *S. dulcamara* (B), a preference for *S. dulcamara* was induced by breeding the larvae from hatching to pupation, and thereupon during ten days after emergence on *S. dulcamara* (see chapter 4.3.1.). In a greenhouse experiments were made to examine whether the rate of oviposition preference for *S. dulcamara* could be increased by breeding on this plant species.

Two series of beetles were reared, one on *S. tuberosum* (AE) and one on *S. dulcamara* (BA). After a regular rate of oviposition was obtained, each series was divided into three groups of 9 beetles. In each series one group was given *S. tuberosum*, one group *S. dulcamara* and one group both plants in competition.

It is remarkable that in the seven days preceding the period of choice the rate of oviposition of *dulcamara*-reared beetles exceeded that of *tuberosum*-reared beetles (fig. 33).

Further it appeared that in the choice experiments by *dulcamara*-reared beetles more eggs were deposited on *S. dulcamara* than were laid on *S. tuberosum*, whereas egg deposition on potato by *tuberosum*-reared beetles outnumbered the deposition on *S. dulcamara*.

The number of eggs counted on *S. tuberosum* and on *S. dulcamara*, offered without competition, do not indicate a stimulation or a suppression of the fecundity by the latter. Apparently suppression by *S. dulcamara*, found in the preliminary experiments, was due to the physiological condition of the plant.

Although these results suggest a conditioning of the oviposition preference on *S. dulcamara*, apparently more factors influence the behaviour of the egg-laying insects as the following experiments will demonstrate.

Two sets of beetles were reared in the laboratory breeding room on potato and on *S. dulcamara* (BA) respectively. After a regular rate of oviposition was established, each set was divided into four groups of three series and was transferred to climate rooms in which the temperature was 15°, 20°, 25° and 30°C respectively. After another six days of acclimatization, one series of each group was given potato, one *S. dulcamara* and one both plants simultaneously.

In fig. 34 the number of eggs of these series during the five experimental days

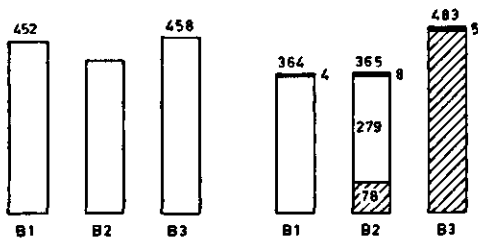
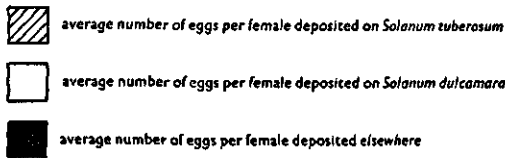
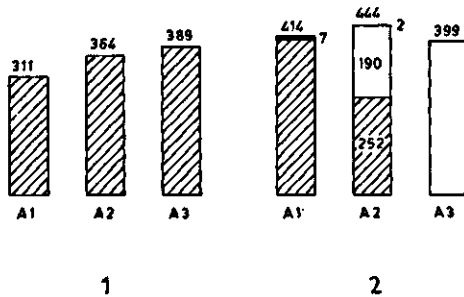


FIG. 33. Oviposition experiment with *Solanum tuberosum* cv 'Eigenheimer' and *Solanum dulcamara*.

1: pre-experimental period (7 days), 2: experimental period (7 days), A: oviposition by beetles reared on *Solanum tuberosum*, B: oviposition by beetles reared on *Solanum dulcamara*.



(18th till 22nd day) is compared with the oviposition during the previous six days on the plant on which these beetles had been reared.

Irrespective of the temperature and independent of the plant species on which the beetles had been reared previously, all groups deposited more eggs on *S. tuberosum* than they did on *S. dulcamara*.

On the 23rd day, when the oviposition experiment was closed, the beetles were starved for 24 hours and given the choice between *S. tuberosum* and *S. dulcamara* for their food. Food consumption during two hours was determined and is expressed in mg dry weight in fig. 34.

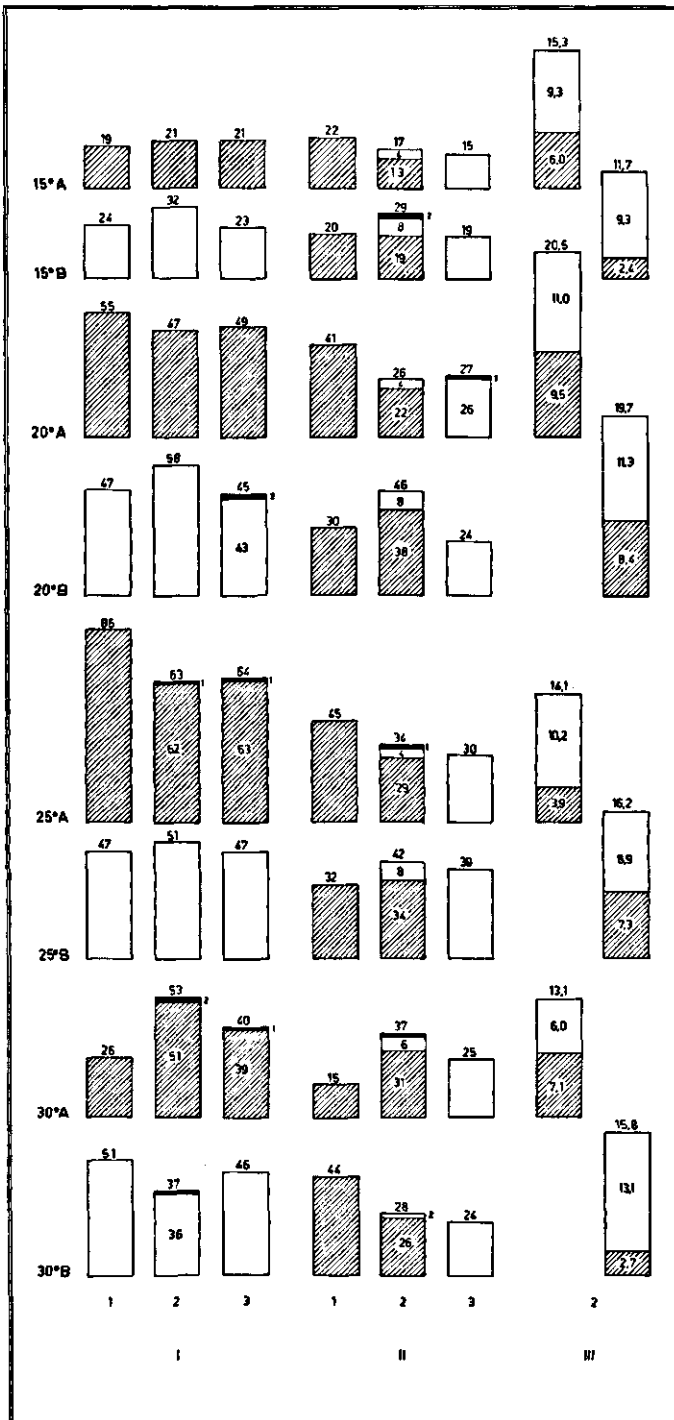
FIG. 34. Fecundity, oviposition choice and food choice at different temperatures of groups of 5 beetles reared on *Solanum tuberosum* (A) or on *Solanum dulcamara* (B);

(for explanation of the diagram, see fig. 33);

I: average number of eggs per female per day during 6 days preceding the experimental period (12th–17th day after hatching);

II: average number of eggs per female per day during 5 days of the experimental period (18th–22nd day after hatching);

III: milligrams dry weight eaten during 2 hours by the beetles of the 2nd groups, after 24 hours of starvation (23rd day after hatching).



Relatively the food intake from *S. dulcamara* by *dulcamara*-reared beetles ($68,7 \pm 7,2\%$) slightly exceeded the consumption from this plant by *tuberosum*-reared beetles ($58,2 \pm 5,6\%$), but all groups consumed more from the leaves of *S. dulcamara* offered simultaneously with leaves of *S. tuberosum*, irrespective of temperature and of the plant species they had been reared on.

The beetles thus treated did not show a food preference for the plant reared on and preferred *S. dulcamara*; nor did they show an oviposition preference for the host they had been reared on as more eggs were deposited on *S. tuberosum*. Only 18,0% of the eggs were deposited on *S. dulcamara* by *dulcamara*-reared beetles and 14,5% by *tuberosum*-reared beetles. However, in both, oviposition and in food choice experiments, the tendency towards *S. dulcamara* was greater in *dulcamara*-reared beetles than in *tuberosum*-reared beetles. Although these results do not demonstrate a marked conditioning, a remanent effect of previous rearing can not be denied.

4.4.3. Further choice experiments with *Solanum luteum*

The strongly positive oviposition response of the beetles to *S. luteum* in the preliminary experiments is of interest. As is shown in the feeding experiments (chapter 4.1.), this plant species does not support larval growth, and is avoided in food choice experiments. In rearing cages the beetles do not feed on it and only nibble a little from the edges of the leaves, from the stem's skin or from the skin of the fruits. They will not eat from it sufficiently, and die from starvation. It is conceivable that the oviposition response to *S. luteum* would be stronger if it were not restrained by food preference for *S. tuberosum*.

The fig. 32 (page 46) represents the total number of eggs deposited by 7 females during the experimental period of 5 days viz. the 20th till the 25th day. This is only the end result and the graph does not give information as to the course of the daily egg production of the females.

The graphs in fig. 35 give information about the course of egg production during the entire observation period. They show that the production of eggs by beetles which have only *S. luteum* at their disposal, ceases at the end of the experimental period and that the main part of the deposition occurs in the beginning. The development of the eggs in the ovaries is apparently due to the consumption of potato during the preceding period.

It is not clear why the egg production of beetles which have *S. tuberosum* and *S. luteum* at their disposal, diminishes during this period to such an extent. Possibly the food consumption of the beetles decreases due to the time spent on *S. luteum* during a part of the day, searching for a site to deposit the eggs.

To find out whether the cessation of the egg deposition on *S. luteum* is due to lack of food or to toxicity of the plant, experiments were performed with four groups of 9 or 10 female beetles:

Group 1 was fed on *S. tuberosum* (AE) during the entire experimental period of 35 days as a control.

Group 2, 3 and 4 were fed on *S. tuberosum* until the 15th day. Starting from that day the beetles of group 2 were given the choice between *S. luteum* and *S.*

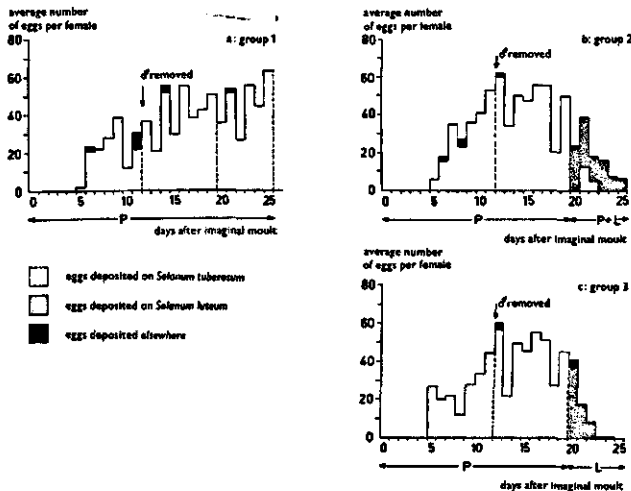


FIG. 35. Oviposition on different food plants during 25 days after hatching, at 25°C, by 3 series of beetles (cf. fig. 32); a: average daily egg production of 7 beetles reared on *Solanum tuberosum* cv 'Vorán' (P); b: average daily egg production of 9 beetles reared on *Solanum tuberosum* (P) and given the choice between *Solanum tuberosum* and *Solanum luteum* (P + L) during 5 days; c: average daily egg production of 9 females reared on *Solanum tuberosum* (P) and offered *Solanum luteum* (L) during 5 days.

tuberosum. It was our aim to provide the beetles with equal amounts of the foliage of each plant species; group 3 and 4 were given only *S. luteum* during 5 days and 10 days respectively, and *S. tuberosum* again from the 15th and the 20th day respectively, until the 35th day.

The graphs of fig. 36 give the average numbers of eggs per female per day. The egg production reached a maximum of about 100 eggs per day round the 10th day, decreased gradually during the rest of the period in groups 1 and 2 and dropped to less than 10 eggs per day in groups 3 and 4 during the period fed on *S. luteum*. Production increased again after replacing *S. luteum* by *S. tuberosum* and within a few days reached a level comparable to groups 1 and 2, demonstrating that *S. luteum* is not toxic and that the decrease of egg production is the result of a decrease of the food intake, due to the non-palatability of *S. luteum*. In all groups the daily egg production at the end of the experimental period was about half the optimum. The average number of eggs per female during the entire period of the experiment is shown in table 3.

The average number of eggs per female during the 10th–35th day of groups 1, 3 and 4 is graphically depicted in fig. 37. The results suggest a negative correlation between the duration of a stay on *S. luteum* and the egg production. If this is the case, the total egg production of group 2, viz. 1001 eggs, is comparable with the expected egg production of a group of beetles given *S. luteum* during a period of 2½ days; 2½ days out of the 24 days of the experiment, means an aver-

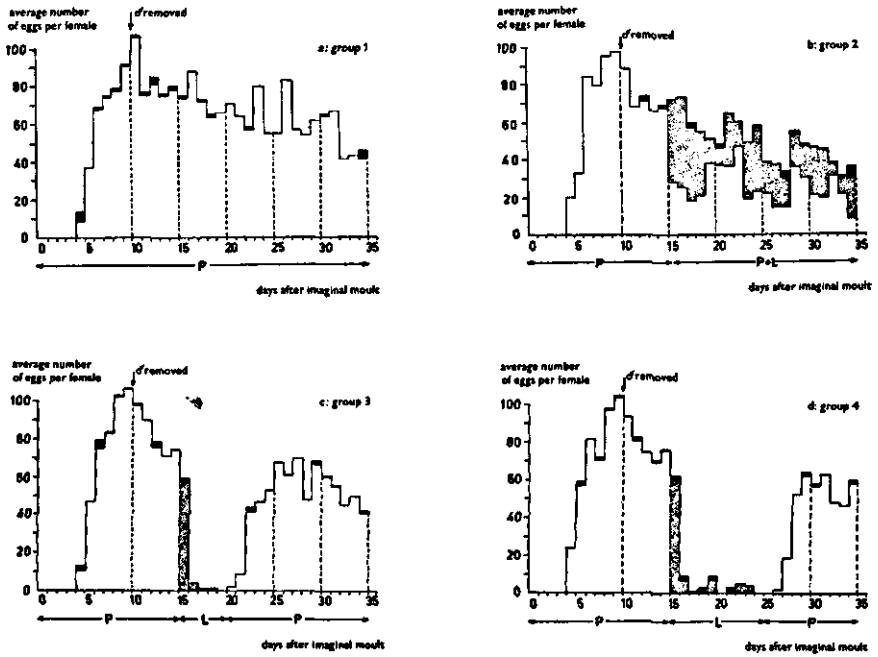


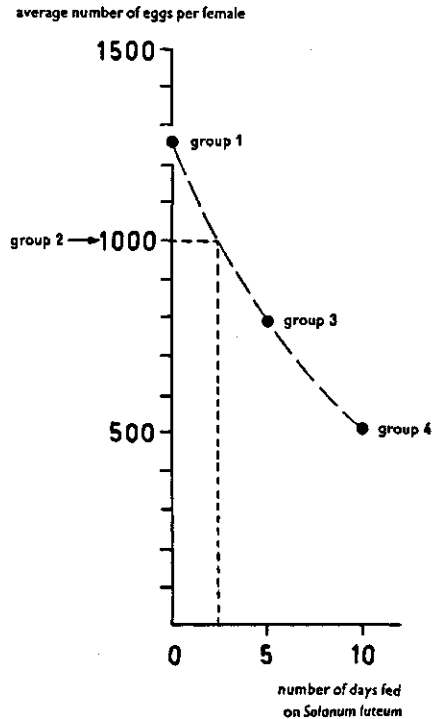
FIG. 36. Fecundity and oviposition choice during 35 days after hatching of 4 groups of beetles: a: average daily egg production of 11 beetles reared on *Solanum tuberosum* cv 'Eigenheimer' (P); b: average daily egg production of 9 beetles reared on *Solanum tuberosum* (P), given the choice between *Solanum tuberosum* and *Solanum luteum* during 20 days (P + L); c: average daily egg production of 10 beetles, reared on *Solanum tuberosum* (P) and offered *Solanum luteum* (L) during 5 days and subsequently given *Solanum tuberosum* again; d: average daily egg production of 8 beetles reared on *Solanum tuberosum* (P) and offered *Solanum luteum* (L) during 10 days and subsequently given *Solanum tuberosum* again; (for explanation of the diagrams, see fig. 35).

TABLE 3. Average number of eggs per female

Days:	0-9	10-35	0-35
Group 1	787	1257	2044
Group 2	767	1001	1769
Group 3	849	793	1643
Group 4	832	510	1342

age of $2\frac{1}{2}$ hours per day. We may assume, therefore, that searching for an oviposition site on *S. luteum* took the beetles an average of $2\frac{1}{2}$ hours per day. It is known (see page 43) that food consumption takes place during 24 hours, though in the dark period at a lower rate (see fig. 30). So it seems reasonable to assume that the beetles spent $21\frac{1}{2}$ hours per day on *S. tuberosum*. This suggests a strong oviposition preference for *S. luteum*. For, although the beetles stayed on *S. tuberosum*

FIG. 37. Influence of the duration of breeding on *Solanum luteum* on the average egg production of the 4 groups of beetles of fig. 36, during the 25 days after removing the males.



7 times as long as on *S. luteum*, the average egg deposition on *S. luteum* (474) was only a few less than on *S. tuberosum* (512). Taking into account the decreased activities during the dark period of 8 hours (see page 43) the beetles stayed on *S. tuberosum* during $13\frac{1}{2}$ hours, which is still 5,4 times longer than their sojourn on *S. luteum*. Thus, although the deposition of eggs on *S. luteum* does not outnumber that on *S. tuberosum* we still may conclude that a strong response to *S. luteum* exists.

4.4.4. Oviposition and age of foliage; food deprivation

Comparing fig. 36b with fig. 35b we conclude that in the preliminary experiments the response to *S. luteum* surpassed the response to *S. tuberosum* (AV) 4,6 times and that in later experiments the number of eggs on *S. luteum* more or less equalled the number of eggs on *S. tuberosum* (AE). To discover whether experimental circumstances have been influencing the results, a new experiment was set up to find out whether the age of *S. luteum*'s foliage determined oviposition preference.

Beetles reared on *S. tuberosum* (AE) were divided into 5 groups. Group 5 was fed on *S. tuberosum* during the entire experimental period of 29 days as a control. The other groups were reared on *S. tuberosum* till the 13th day. From the 14th till the 29th day group 1 was given equal amounts of foliage surface of *S. tubero-*

sum and of senescent leaves of *S. luteum*. Group 2 was treated as described for group 1 except that the foliage of *S. luteum* was derived from younger plants. Group 3 was given foliage from the younger plants from the 14th day till the 18th, whereupon they were fed on *S. tuberosum*. To determine whether the cessation of the egg production was indeed caused by lack of food and not by other influences, group 4 was starved during the period in which group 3 was fed on *S. luteum*. Only an oak twig was offered to sit on. A soaked plug of cottonwool enabled the beetles to drink water; after the 18th day *S. tuberosum* was supplied again.

The results are given in the graphs of fig. 38. Again the maximum egg production of all groups was obtained on about the tenth day, whereupon, in the control group the production decreased gradually. Of the total amount of eggs laid between the 13th and the 29th day, 37% was deposited on the old leaves of *S. luteum* by group 2, whereas 63% on *S. tuberosum*; only a few eggs were deposited elsewhere, viz. 0,5% (table 4). Group 2 deposited $\pm 41\%$ of the eggs on the younger leaves of *S. luteum*, $\pm 55\%$ on *S. tuberosum* and $\pm 4\%$ elsewhere (table 4). In group 3 a rapid decline of the egg production to nearly zero within 3 days was noted when *S. luteum* was offered. Given *S. tuberosum* again, a recuperation of the egg production occurred within 6 days. In group 4 starvation caused an immediate cessation of the egg production. When *S. tuberosum* was offered again, oviposition was resumed only on the 3rd day. Recovery of normal production took 9 days.

TABLE 4. Average number of eggs per female (14th–29th day)

	Group 1 (AE + L _{old})	Group 2 (AE + L _{young})
<i>S. tuberosum</i>	607 (62,70%)	479 (55,06%)
<i>S. luteum</i>	356 (36,77%)	354 (40,69%)
elsewhere	5 (0,51%)	37 (4,25%)
total	968	870

This experiment did not solve the problem why such a marked oviposition preference for *S. luteum* occurred in the preliminary experiments. Possibly a difference in plant breeding method caused these results. Still it must be emphasized that it is remarkable that so many eggs were deposited on *S. luteum*, though the beetles show a feeding preference for *S. tuberosum* and hardly feed on *S. luteum* at all. In our opinion this fact points to an oviposition preference for *S. luteum*, which is diminished by the food preference for *S. tuberosum*.

In all groups the daily production of eggs at the end of the experimental period is about $\frac{2}{3}$ of the maximum which falls on the tenth day. Thus influences, due to feeding on *S. luteum* and even to deprivation of food, on oogenesis are reversible by feeding on *S. tuberosum*. The total number of eggs per female of the different groups in the entire period is given in table 5 and in fig. 39.

Again we may conclude that dwelling on *S. luteum* is responsible for a decreased food intake and, consequently, diminishes the egg production. On the

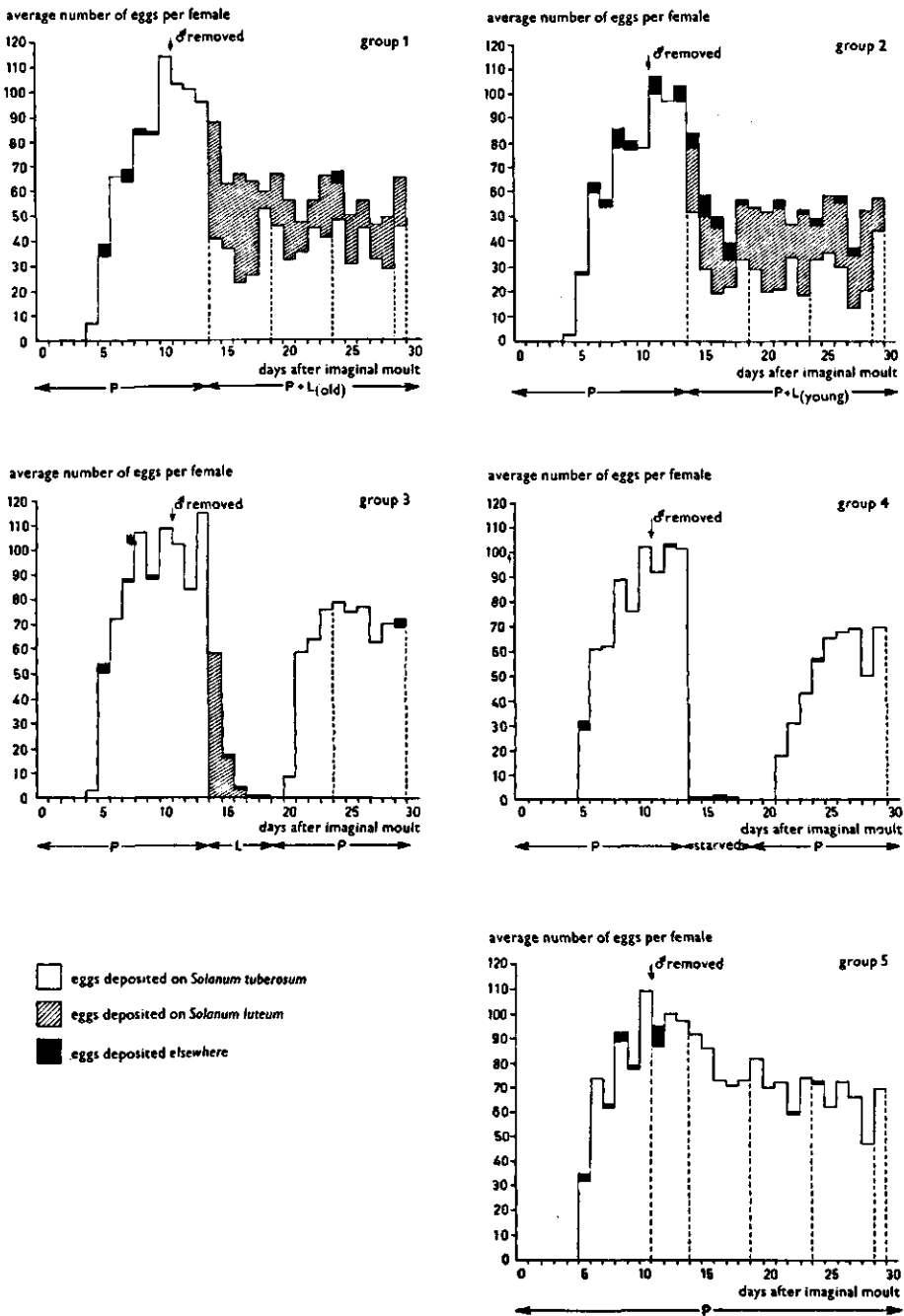


FIG. 38. Fertility and oviposition choice during 29 days after hatching of 5 groups of beetles: group 1: average daily egg production of 9 beetles reared on *Solanum tuberosum* cv 'Eigenheimer' (P), given the choice between *Solanum tuberosum* and old foliage of *Solanum luteum* ($P + L_{(old)}$) during 16 days; group 2: average daily egg production of 9 beetles reared on *Solanum tuberosum* (P), given the choice between *Solanum tuberosum* and young foliage of *Solanum luteum* ($P + L_{(young)}$) during 16 days; group 3: average daily egg production of 9 beetles reared on *Solanum tuberosum* (P), and provided with young foliage of *Solanum luteum* (L) during 5 days and subsequently given *Solanum tuberosum* again; group 4: average daily egg production of 10 beetles reared on *Solanum tuberosum* (P), and starved during 5 days; group 5: average daily egg production of 9 beetles reared on *Solanum tuberosum* (P).

TABLE 5. Average number of eggs per female during the period mentioned in fig. 38.

Day:	0-13	14-29	0-29
Group 1	745	1142	1887
Group 2	764	968	1732
Group 3	705	870	1574
Group 4	822	725	1547
Group 5	719	474	1193

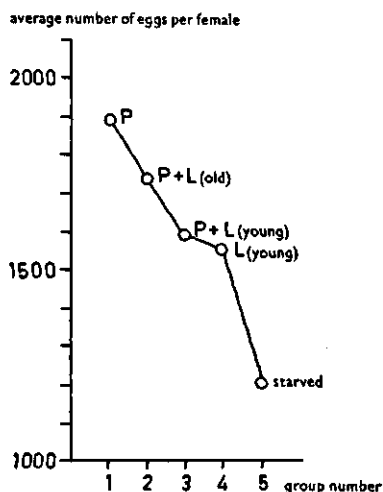


FIG. 39. Average egg production per female during the experimental period of 29 days of the 5 groups mentioned in fig. 38.

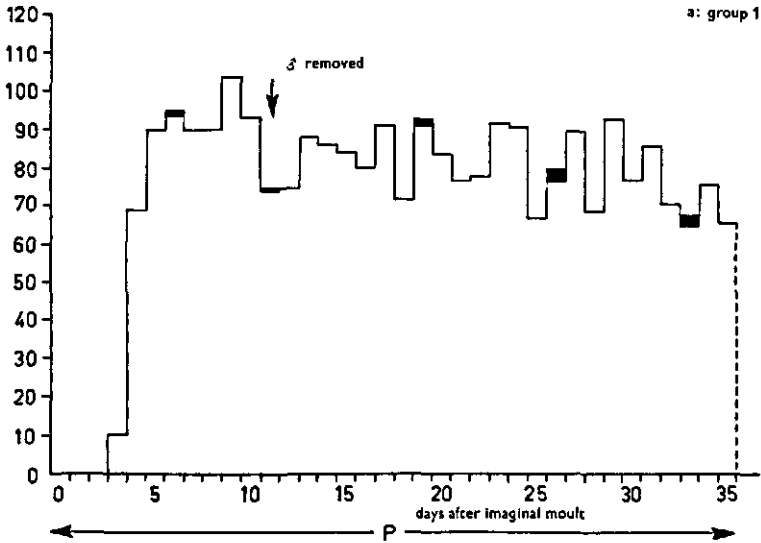
other hand, *S. luteum* probably supplies some food to the beetles, for the decrease in productivity of beetles living on *S. luteum* is less rapid than that of starved beetles.

To determine whether the decline of the egg production, when fed on *S. tuberosum* and *S. luteum* both, is reversible by feeding on *S. tuberosum*, a final experiment has been set up as follows:

Of 2 groups of 10 beetles, reared on *S. tuberosum* (AE), one was offered only *S. tuberosum* and the other both *S. tuberosum* and *S. luteum* from the 17th till the 26th day (see fig. 40). Here too a decrease of the egg production was noted, though sufficient food was presented in the form of *S. tuberosum*. When offered only *S. tuberosum* again, on the 27th day an increase was noted and the end result was entirely in accordance with the egg production of the controls.

Given the choice between *S. tuberosum* and *S. luteum*, 54,6% of the eggs was deposited on the latter and 43,9% on *S. tuberosum*. In comparison with the former experiment, in which young and old leaves of *S. luteum* were offered, the plants of this experiment were very old and senescent. Thus, the older the *S. luteum* plants, the more they are preferred for oviposition. This may be the solution of the problem posed by the results of our preliminary experiments.

average number of eggs per female



average number of eggs per female

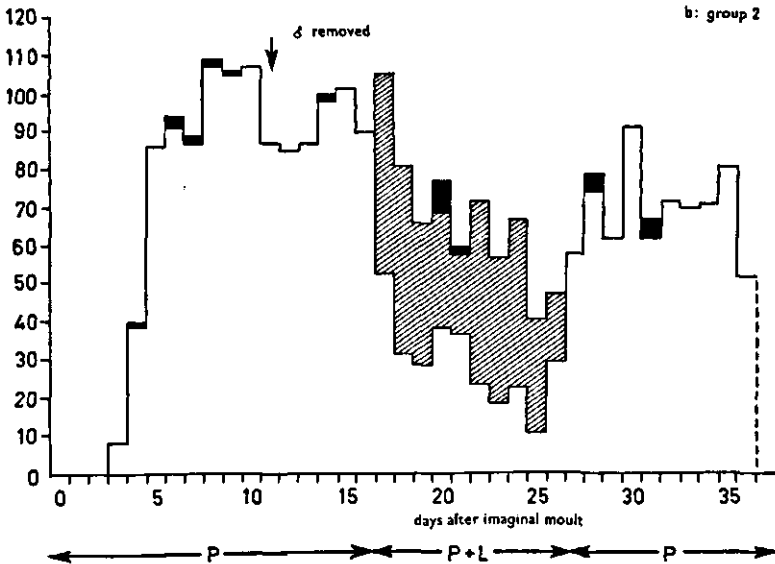


FIG. 40. The reversibility of the fecundity decrease due to *Solanum luteum*:

a: average daily egg production of 10 beetles reared on *Solanum tuberosum* cv 'Eigenheimer' (P);

b: average daily egg production of 10 beetles reared on *Solanum tuberosum* (P), given the choice between *Solanum tuberosum* and *Solanum luteum* (P + L) during 10 days, and subsequently provided *Solanum tuberosum* again; (for explanation of the diagrams, see fig. 38).

4.4.5. Summary of the results of the experiments on oviposition

Summarizing the results of the oviposition experiments, we conclude that there are indications that breeding on *S. dulcamara* increases the oviposition preference to that plant species, though the physiological condition of the host plant is of dominant influence on the oviposition choice; it seems to be of more importance than the other experimental circumstances.

Food intake and starvation immediately affect the production of egg laying females; cessation of the production by starvation or by rearing on the unpalatable *S. luteum* is reversible by feeding. This again proves that *S. luteum* is not toxic.

The beetles have a marked preference for *S. luteum* as an oviposition substratum when given the choice between this plant and *S. tuberosum*; this preference is apparently decreased by the food preference for *S. tuberosum*. The older the *luteum* plants, the greater their attraction. A daily period spent on *S. luteum* causes a diminished food intake and, by that, a decreased egg production, though a sufficient supply of food is available. Apparently time is a limiting factor: removal of the *luteum* leaves raises the production.

4.5 ORIENTATION TO THE HOST PLANT

4.5.1. Screen test

When starved beetles, kept separately in a petri dish, were offered a potato leaflet, usually a stereotype behaviour was observed, viz. after a short period of immobility, the beetles began to sway their antennae with an increased frequency; then they turned round and walked in a straight line to the leaf (fig. 41).

SCHANZ (1953) demonstrated the role of the antennae in the Colorado beetles' final orientation to the food plant. Normal animals with the eyes painted, found small pieces of potato leaf in an average time of four minutes. With three segments of the antennae missing from both sides, blinded beetles took ten minutes; with four or more segments missing they were unable to find the bait. CHIN (1950) and DE WILDE (1958) found that olfactory information guided the Colorado beetle larva to the food plant.

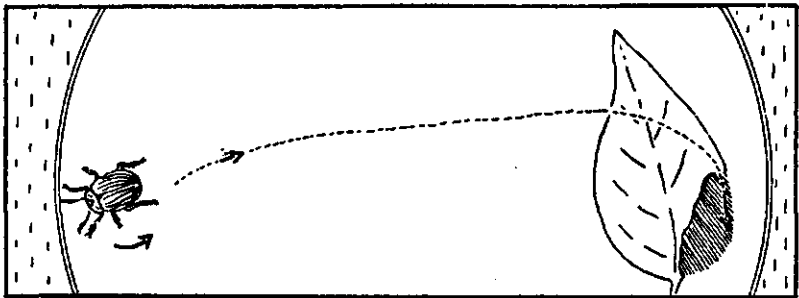


FIG. 41. A Colorado beetle in a petri dish, offered a leaflet of *Solanum tuberosum*.

The observed behaviour and the information quoted above led us to the following hypothesis: in starved Colorado beetles searching for food is elicited by odour emanating from the plant, and is guided by optical stimuli.

To test this hypothesis a series of screen tests were performed as described in chapter 3.6.1. The series consisted of the following situations offered to the beetles (fig. 42a):

Situation I: the two spaces underneath the gauze empty (control-situation).

Situation II: one of the two spaces underneath the screen contained cut pieces of potato leaves in such a way that the beetles could not touch the foliage through the gauze. In this half of the glass box the 'arena' emanated the odour of potato foliage. This half was supposed to provide olfactory stimuli.

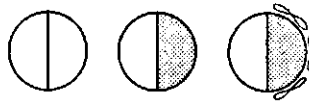
Situation III: three leaves of potato plants, stuck with cellotape outside the wall

a: screen test



Situation	I	Ia	II	III	IV	V	Va
Number of pairs of beetles tested	70	20	50	80	60	84	20
Total number of beetles counted in the two halves of the arena	1400:1400	556:244	862:1138	1633:1567	1450:950	1550:1810	537:263
Average number of beetles per test counted in the two halves of the arena	20,0:20,0	27,8:12,2	17,2:22,8	20,4:19,6	24,1:15,9	18,5:21,5	26,85:13,55
P	-	< 0,01	< 0,01	-	< 0,01	< 0,01	< 0,01

b: olfactometer test



Situation	VI	VII	VIII
Numbers of pairs of beetles tested	10	10	10
Total number of beetles counted in the two halves of the arena	196:204	146:254	116:284
Average number of beetles per test counted in the two halves of the arena	19,6:20,4	14,6:25,4	11,6:28,4
P		< 0,01	< 0,01

FIG. 42. Screen test and olfactometer test.

of one half of the glass box provided optical stimuli, while no leaves occupied the spaces underneath the gauze.

Situation IV: a combination of situations II and III in such a way that the optical component was present in the section of the arena opposite to the half provided with cut leaves.

Situation V: resembled situation IV with the exception that both spaces were equipped with cut leaves.

Derived from this series a second one was composed:

Situation Ia: the glass box was surrounded by a cylinder painted black and white, providing one half of the arena with a white and the other with a black wall (see fig. 42a). The spaces underneath the screen were empty.

Situation Va: resembled Ia and differed from this by the presence of cut leaves in both spaces underneath the gauze.

The results are given in fig. 42a. A statistical analysis has been made of these results according to the χ^2 test.

An equal distribution is to be seen in situation I, whereas in situation Ia significantly more beetles were counted in the white half of the glass box. Apparently reaction to the light distribution caused the beetles, positive phototactic as they are, to dwell longer in this part of the arena.

In situation II, in the part with the cut leaves under the gauze, the beetles outnumber those of the other part.

No statistical differences were observed between the numbers in the two halves in situation III.

In situation IV in the 'olfactory' part more beetles were scored than in the 'optical' half.

In situation V, however, more beetles were seen in the 'optical' part. The olfactory stimuli in each part were equal and, evidently, the optical stimuli attracted the beetles.

The results in situation Va, in which the number of beetles in the white half exceeded the number in the black part, suggest that it is not the light intensity reflecting from the leaves, but rather their shapes, and may be, in addition, also the colour that influences the behaviour. This may also explain why there was a difference between Ia and III. Although there was a difference in shade between the dark halves of Ia and III, on account of the results in situation Ia the expectation in situation III is to find more beetles in the part of the glass box without leaves outside the wall, than in the other part. In situation Ia apparently the beetles avoided the dark half due to positive phototaxis, whereas in situation III this phototaxis was more than compensated for by the shape or colour of the leaves (in larvae a true colour vision was demonstrated by DE WILDE and PET, 1957).

Situation IV, at first sight, seems in contradiction with our supposition of the attractivity of optical stimuli and it disaffirms our hypothesis as do the results of situation II. Here we must discuss the merits of the method used:

Active beetles will walk round and round the arena and a 'choice' made by them will never lead to a 'consummatory act' in the ethological sense (TINBERGEN,

1951). Consequently the motivation for searching for food will never be removed. Walking around on the screen the beetles will return into the initial situation. This explains why in the choice situation of the screen test we must not expect large differences between the numbers of beetles in the two halves of the glass box. We may nevertheless conclude from the results of our screen-test experiments described above that in the situations created in the glass box obviously olfactory information is of prime importance and is more effective in orientating the beetles' behaviour than optical stimuli. Only in cases where no olfactory gradient can play a role, visual information leads the beetles in their choice.

The hypothesis has to be adapted: in starved Colorado beetles searching for food is elicited by odour emanating from the plant and is guided by both optical and olfactory stimuli. Both types of stimuli may either act separately or in conjunction.

4.5.2. Olfactometer test

The results mentioned above are confirmed in the olfactometer test of which the experimental set up is described on page 24 and a diagram is given in fig. 9. The series of choice situations was as follows:

Situation VI: both air flows without odour;

Situation VII: one air flow led through foliage of *S. tuberosum*;

Situation VIII: resembled to situation VII and differed from this that leaves of *S. tuberosum*, stuck with cello tape outside the wall of the half of the cylinder emanated with odour, provided optical stimuli. The results are given in fig. 42b.

An equal distribution is observed in situation VI, whereas in situation VII significantly more beetles were counted in the 'potato-scent' half of the arena, apparently due to olfactory stimuli. In situation VIII this olfactory effect is enhanced by optical stimuli. This suggests that odours emanating from the potato plant elicit searching behaviour for food or, otherwise, act as an arrestant for the beetle.

5. DISCUSSION AND CONCLUSIONS

To investigate the dynamics of host plant preference of *Leptinotarsa decemlineata* SAY, it was necessary to apply several criteria. As an introduction to the research experimental rearing of this beetle on several plants was conducted. Larvae as well as beetles showed preference for several plants as food, which was deduced from the amount of food consumed. Other criteria for the suitability of plants as hosts were the rate of larval development, larval mortality and fecundity.

Although certain plant species (e.g. *Solanum lycopersicum*) provide excellent food, they are only consumed when no other food is available. Other plants (e.g. *Solanum luteum*) are never accepted, even when starvation is the only alternative. It was found that *Solanum tuberosum* was preferred above the other food plants, but *Solanum dulcamara*, *Solanum rostratum* and *Solanum carolinense* are readily eaten too. As mortality during the larval period by breeding on the last mentioned plants outnumbered mortality on *Solanum tuberosum* this species yields the most promising prospects for survival of the Colorado beetle, though in our experiments little variability in the duration of larval development on different foodplants occurred; it was influenced by experimental circumstances. *Solanum lycopersicum* was only eaten when nothing else was available; the insect will manage to survive on this plant species if the right cultivar is chosen (cf. BUHR, 1956, 1961). *Solanum luteum* and *Solanum nigrum* were refused and but rarely one larva out of hundred survived and moulted into a beetle; these plant species are not to be considered as possible host plants for *Leptinotarsa decemlineata*. *Solanum luteum* is not toxic as was demonstrated by alternate feeding with *Solanum tuberosum*. Feeding experiments with *Solanum tuberosum* leaves impregnated with juices from *Solanum luteum* leaves, suggest that the latter possesses rejective properties.

There is no indication that the preference for *Solanum dulcamara* is conditioned by rearing on this plant. Results of our preliminary experiments (DE WILDE et al., 1960), suggesting the contrary, probably were evoked by experimental circumstances such as the strain and the physiological condition of the plant material used and the ambient temperature: young foliage proved to be preferred to senescent leaves; when offered simultaneously, at lower temperatures (below 25°C) *Solanum tuberosum* appeared to be favoured as food and at higher temperatures (above 25°C) *Solanum dulcamara* was preferred. The ambient temperature, however, did not influence the consumption from rejected plants. It was possible to alter food preferences by inbreeding during several generations on *Solanum dulcamara*, which plant became favoured by the beetle. Probably by rearing them on *Solanum rostratum*, a similar shift in preference can be induced. The shift, however, could be a result of selection rather than of adaptation. The preference for *Solanum tuberosum* was not altered by rearing either on *Solanum rostratum* or on *Solanum carolinense*.

We conclude that, in *Leptinotarsa decemlineata*, food preferences are innate but can be modified by variety or strain, by the physiological condition of the plant and by ambient temperature.

In comparison with *tuberosum*-reared beetles, fecundity of the Colorado beetles reared on various food plants, was depressed slightly by *Solanum dulcamara*, more by *Solanum lycopersicum* and heavily by *Solanum luteum*. These results agree with the food preference and with the conclusions drawn on survival prospects on these plants mentioned above. The fecundity decline is caused by the decrease in food consumption, due to the apparently less palatable properties of these plants, and is reversed by feeding on a preferred plant, even when the decline has been the result of starvation.

Solanum tuberosum is preferred as a substratum for oviposition when compared with *Solanum dulcamara* or with *Solanum lycopersicum*. Though *Solanum luteum* suppresses fecundity and is rejected as food, more eggs were deposited on this plant than on *Solanum tuberosum* when offered simultaneously. It is conceivable that the oviposition response to *Solanum luteum* would be even stronger if it were not restrained by the food preference for *Solanum tuberosum*. In the choice situation the daily period spent on *Solanum luteum* causes a diminished food intake and, as a consequence, a decreased egg production, even though sufficient supply of adequate food is available. Apparently time is a limiting factor as the removal of *Solanum luteum* increases the fecundity.

The discrepancy between food and oviposition preference leads us to the conclusion that oviposition preference is not a consequence of food preference and that these responses are based on different mechanisms. This agrees with analogous observations of HSIAO and FRAENKEL (1968d) who found that *Solanum nigrum* is preferred for oviposition to *Solanum tuberosum* though the former is not eaten by either adults or larvae.

Oviposition preference appeared to be independent of temperature (i.e. no relation between temperature and preference was observed), and could not be conditioned by previous breeding, at least in experiments with *Solanum dulcamara*. This is in agreement with the conclusions mentioned above.

The preference for *Solanum luteum* as an oviposition substratum increased as the plants grew older. Apparently the characteristics promoting the response intensify in older plants.

Although *Leptinotarsa decemlineata* favours *Solanum luteum* as an oviposition substratum, this does not mean that this behaviour is biologically fatal to the species. Whereas a period of feeding precedes the oviposition period, and the main period for lengthy flight in all probability is during the pre-oviposition period (HASKELL, 1966) as the gravid female does hardly succeed in flying, oviposition normally will occur on the preferred food plant on which the females complete their maturation feeding. It is, therefore, not to be expected that oviposition preference will interfere with food preference.

In starved Colorado beetles, searching for food is elicited by odour emanating from the plant and is guided by both optical and olfactory stimuli. Both

types of stimuli may either act separately or in conjunction. These observations, however, do not justify any conclusions concerning longer range attraction in the field, as is also emphasized by KENNEDY (1965). That olfactory stimuli do play a dominant role in host plant finding is demonstrated by DE WILDE et al. (1969); moreover they are an important factor in host plant recognition and have a strong effect as an arrestant.

Although it is not impossible to modify the host plant preference of the Colorado beetle by external factors, we conclude that a process of habituation or 'learning' is not responsible for the host plant affinity of *Leptinotarsa decemlineata* SAY.

6. SUMMARY

Host plant choice, suitability of and conditioning to the host in *Leptinotarsa decemlineata* SAY were studied under controlled conditions.

The literature on historical and geographical distribution of the Colorado beetle has been reviewed and an extensive survey is given of the literature on food plant range, host plant selection, orientation to the host, host plant recognition and conditioning of host plant preference concerning *Leptinotarsa decemlineata*.

Definitions of host plant relationship and several other criteria are discussed. In this thesis 'feeding preference for a given plant' means that in a choice test, the quantity eaten from this plant exceeds the quantity consumed from each of the other plants. An 'oviposition preference for a given plant' means that the number of eggs deposited on this plant outnumbers the oviposition on any other plant at the disposal of the insect.

All experiments were performed with insects reared in the laboratory; most of the plants used were grown in a greenhouse.

The suitability of the plants to function as host was determined by rearing beetles *ab ovo* on *Solanum tuberosum*, *Solanum dulcamara*, *Solanum luteum*, *Solanum nigrum* and *Solanum lycopersicum*. The results appeared to coincide with the preference for food. *Solanum tuberosum* gives best results and is preferred to *Solanum dulcamara*, which gives satisfying results (as do *Solanum carolinense* and *Solanum rostratum*) and is readily eaten too. *Solanum lycopersicum* is normally avoided and only eaten when nothing else is available; it yields poor rearing results, which differs with different strains used.

Solanum luteum and *Solanum nigrum* are avoided even when starvation is the only alternative. The avoidance of *Solanum luteum* apparently is caused by rejective properties and not by lack of phagostimulants or toxic compounds. This is shown by oviposition experiments, by alternate feeding, by sandwich tests and by food choice experiments with leaves impregnated with juices of *Solanum luteum*.

The duration of larval development on the different food plants is not very variable and is dependent on experimental circumstances (e.g. temperature and physiological condition of the plants).

Food choice experiments with larvae and adult beetles reared on *Solanum tuberosum* or one of the other experimental plants, gave no indication that pre-imaginal experience had any influence on host preference of the insect. *Solanum tuberosum* was preferred to all other plants used. Food choice was greatly influenced by the condition of the plant, the plant variety and the test conditions.

With increasing temperatures the rate of food intake also increases, but more so with *Solanum dulcamara* than with *Solanum tuberosum* when offered simultaneously; *Solanum tuberosum* is preferred at lower temperatures, *Solanum dulcamara* at higher temperatures. This was true for both newly emerged and

mature beetles, reared previously on either of these plants. Ambient temperature had no influence on the consumption of rejected plants. These effects as well as other factors influencing the food choice experiments have been discussed.

Inbreeding on *Solanum dulcamara* resulted in a food preference for this plant species after eight and more generations.

In screen tests and in olfactometer tests, performed with female beetles, besides olfactory stimuli, optical stimuli were introduced also. Both types of stimuli elicited searching behaviour, either separately or in conjunction and play a most important role in host plant recognition as well as in host plant finding. However, no conclusions about longer range attraction can be made yet.

In oviposition preference experiments solitary female beetles were given the choice between *Solanum tuberosum* on the one hand and one of the experimental plants on the other. In control series the fecundity of the beetles was tested by rearing on both test plants separately. In comparison with *tuberosum*-reared beetles, fecundity of the Colorado beetles, reared on various food plants (as measured by the number of eggs deposited), was slightly depressed by *Solanum dulcamara*, more so by rearing on *Solanum lycopersicum* and heavily by rearing on *Solanum luteum*. It is very probable that the decline of fecundity is caused by a decrease of food consumption, owing to the apparently less palatable properties of these plants; renewed fecundity can be achieved by giving the beetles suitable food. *Solanum tuberosum* appeared to be preferred for oviposition to *Solanum dulcamara* and *Solanum lycopersicum*; *Solanum luteum*, however, was preferred to *Solanum tuberosum* as an oviposition substratum, though this plant suppresses the fecundity and is avoided as food plant. Oviposition response to *Solanum luteum* could have been even stronger when the preference for *Solanum tuberosum* as food would not have interfered. In the choice experiments there is probably a decreased food intake and consequently a decreased egg production due to the daily period spent on *Solanum luteum*. Time appeared to be a limiting factor and, indeed, removal of *Solanum luteum* caused an increased fecundity. The conclusion is drawn that in host plant choice the selections of food and of oviposition substratum are based on two different mechanisms. Furthermore we conclude that this discrepancy will not be fatal for the insect, as a period of maturation feeding precedes the reproductive phase and consequently food plant choice by the adult beetle will determine the oviposition choice.

Although it is possible to modify the host plant preference of the Colorado beetle, we conclude that habituation or 'learning' is not responsible for the host plant affinity of *Leptinotarsa decemlineata* SAY.

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8. SAMENVATTING

Dit proefschrift handelt over enige betrekkingen tussen de Coloradokever (*Leptinotarsa decemlineata* SAY) en zijn waardplant. Van deze kever werd, onder laboratorium-omstandigheden, een studie gemaakt van zijn waardplantkeuze. Hierbij vroegen we ons onder meer af, of de voorkeur van de kever steeds naar de aardappelplant als voedsel en als ondergrond voor de ei-afzetting uitgaat; of de ei-afzetting van de kevers ook op andere planten dan op aardappels plaats vindt; of deze voorkeur aangeboren is of door voorafgaande ervaring beïnvloed wordt (hoe is de voedselvoorkeur van een kever, die op een bepaalde soort voedselplant uit het ei komt?); of de keuze van een plant als voedsel en als ondergrond voor de ei-afzetting aan elkaar ondergeschikt zijn (legt de kever eieren op de plant van zijn voedselvoorkeur of eet hij de plant waarop hij bij voorkeur zijn eieren legt?); of uitwendige factoren (vnl. temperatuur) een rol spelen bij de waardplantkeuze.

Literatuurgegevens worden besproken over de geschiedenis van de geografische verspreiding van de Coloradokever, die voor het eerst is beschreven in 1823 in het Oosten van de Verenigde Staten, waar hij waarschijnlijk leefde op stekelnachtschade ('buffalo bur', *Solanum rostratum*). Tussen 1850 en 1870 verspreidde hij zich over het gehele gebied van dat land en ontwikkelde zich tot een ernstige bedreiging van de aardappelcultuur. Sinds 1920 komt hij ook in Europa voor, waarin hij zich eveneens snel verbreedde, behalve in Groot Brittannië, waar hij niet voorkomt.

De belangrijkste literatuur is besproken over een reeks van planten van het geslacht Nachtschade (*Solanum*), waartoe alle voedselplanten van de Coloradokever horen en die voor dit onderzoek van belang zijn; over de waardplantkeuze van de Coloradokever, met name over het vinden en het 'herkennen' van de waardplant; en over de invloed van voorafgaande ervaring op de waardplantkeuze in het algemeen en die van de Coloradokever in het bijzonder (Hoofdstuk 1).

Voordat werd ingegaan op de methoden van onderzoek en het gebruikte planten- en dierenmateriaal, worden enkele begrippen, als voedselpreferentie en ovipositiepreferentie, gedefinieerd en enkele maatstaven daarvoor besproken. Van voedselpreferentie voor een bepaalde plant wordt gesproken wanneer van deze plant een grotere hoeveelheid wordt gegeten dan van elk der andere planten, die tegelijk worden aangeboden. Ovipositiepreferentie voor een bepaalde plant wil zeggen, dat op deze plant een groter aantal eieren wordt gelegd dan op elk der andere planten, die op dat moment voor de kever beschikbaar zijn (Hoofdstuk 2).

Het insectenmateriaal was afkomstig uit de laboratoriumkweek; de meeste proefplanten werden geteeld in een daartoe bestemde kas (Hoofdstuk 3).

Door, vanaf het uitkomen uit het ei, larven te kweken op het loof van aardappel (*Solanum tuberosum*), bitterzoet (*Solanum dulcamara*), dons nachtschade (*So-*

lanum luteum), tomaat (*Solanum lycopersicum*) en zwarte nachtschade (*Solanum nigrum*) is nagegaan of deze planten voor de Coloradokever geschikt zijn als voedselplant; de uitkomsten hiervan werden vergeleken met de voedselpreferentie. Het beste laat de kever zich kweken op aardappelloof, waarvoor hij ook de grootste voorkeur heeft. Op bitterzoet is de sterfte ongeveer drie keer zo hoog; het wordt na aardappel het liefst gegeten, terwijl stekelnachtschade ('buffalo bur', *Solanum rostratum*) en 'horse nettle' (*Solanum carolinense*) ongeveer even aantrekkelijk zijn. Tomaat is geen aantrekkelijke plant en er wordt weinig van gegeten als er een keuze is. Ondanks een hoog sterfte-percentages (vijf keer hoger dan op aardappel) is het mogelijk op deze plant kevers te kweken. Van dons-nachtschade en van zwarte nachtschade wordt nauwelijks of niet gegeten, ook al is de hongerdood het enige alternatief; niettemin ontwikkelden soms enkele larven zich tot kevers.

Dat het versmiden van deze plantensoorten kennelijk berust op afweerstoffen van de planten en niet op gebrek aan vraatstimulerende bestanddelen (phagostimulantia) of op giftigheid, bleek respectievelijk uit wisselproeven (waarbij afwisselend om de andere dag aardappelloof en loof van dons-nachtschade werden aangeboden, hetgeen de levensduur rekte in vergelijking met die van larven, die om de andere dag vastten) en uit 'sandwich'-proeven en keuzeproeven waarbij aardappelloof werd geïmpregneerd met sap van dons-nachtschade.

De ontwikkelingsduur van de larven op de verschillende voedselplanten loopt niet sterk uiteen en is afhankelijk van de kweekomstandigheden.

Voedselkeuzeproeven met larven en met kevers, die op verschillende voedselplanten werden gekweekt, gaven geen enkele aanwijzing, dat voorafgaande ervaring invloed heeft op de voedselvoorkeur. Aardappel bleek in alle gevallen favoriet. Wel bleken de fysiologische conditie en de variëteit van de plant en ook de temperatuur, waarbij de kever tot stand kwam, van betekenis. Naarmate de temperatuur steeg werd door de kevers meer voedsel opgenomen. De toename van de vraat van bitterzoet nam echter sterker toe dan die van aardappelloof, wanneer deze samen aangeboden werden. Bij temperaturen beneden $\pm 25^{\circ}\text{C}$ werd aardappel geprefereerd, boven deze temperatuur daarentegen bitterzoet. Pas ontpopte kevers gedroegen zich hierin niet anders dan kevers, die hun rijpingsvraat voltooid hadden. Ook maakte het geen verschil of deze kevers waren gekweekt op aardappelloof of op loof van bitterzoet. De voedselpreferentie bleek dus temperatuurafhankelijk; of dit toegeschreven moet worden aan eigenschappen (die met de temperatuur veranderen) van de kever of van de planten is ter discussie gebracht. Op de hoeveelheid, die van onaantrekkelijke voedselplanten werd gegeten, had de temperatuur geen invloed.

Inteelt gedurende enige generaties van dieren, die op bitterzoet werden gekweekt, veroorzaakte een voedselvoorkeur voor deze plant, die waarschijnlijk eerder aan selectie dan aan adaptatie moet worden toegeschreven.

Gebleken is, dat de voedselopname 's nachts sterk afneemt en dat het dag- en nachtrythme wordt gereguleerd door de licht/donker-periode. Verandert men de fase hiervan, dan ijlt de fase van de vreetactiviteit nog minstens 24 uur na. Voedselkeuzeproeven dienen daarom bij dag te worden verricht, bij een con-

stante licht/donker-periode. Op welk deel van de dag (licht-periode) dit gebeurt is niet van belang daar de voedselopname gelijkelijk over deze periode bleek te zijn verdeeld (fig. 30).

In 'screen tests' (fig. 8) en olfactometerproeven (fig. 9) werden naast geurprikkelers ook optische prikkels gegeven aan vrouwelijke kevers. Beide afzonderlijk bleken zoekgedrag op te wekken. Samen werkten zij versterkt. Hoewel hieruit geen gevolgtrekkingen gemaakt kunnen worden over het vinden van de waardplant over een grotere afstand, mag uit deze experimenten worden geconcludeerd, dat geur- en gezichtszin beide een belangrijke rol spelen bij het vinden en 'herkennen' van de waardplant.

Aan individuele wijfjes werd voor de ei-afzetting de keuze gegeven tussen aardappelloop enerzijds en loof van bitterzoet, van donsnachtschade of van tomaat anderzijds (ovipositiekeuzeproeven, zie fig. 7). In contrôle-series werd steeds de vruchtbaarheid van de kevers op beide plantensoorten vastgesteld. Deze bleek door het kweken op bitterzoet, donsnachtschade of tomaat af te nemen en wel het sterkst op donsnachtschade, minder op tomaat en het minst op bitterzoet. Deze afname is reversibel en werd, zo concluderen wij, waarschijnlijk veroorzaakt door een verminderde voedselopname als gevolg van de geringere voedselvoorkeur voor deze planten. Het loof van aardappel werd geprefereerd als ondergrond voor de ei-afzetting boven dat van bitterzoet of tomaat; op donsnachtschade werden daarentegen meer eieren afgezet dan op aardappelloop, hoewel deze plant de vruchtbaarheid vermindert en als voedsel niet geaccepteerd wordt. De ei-afzetting op donsnachtschade zou wellicht nog groter zijn geweest wanneer de voedselvoorkeur voor aardappelloop hierin geen rol speelde. De afname van de vruchtbaarheid van de kevers, die de keuze hadden tussen loof van aardappel en van donsnachtschade, werd waarschijnlijk veroorzaakt door een verminderde voedselopname, doordat de kevers een deel van de dag doorbrachten op laatstgenoemde plant, voor welke zij een ovipositievoorkeur hebben. Tijd is hier kennelijk een beperkende factor: werd de donsnachtschade weggenomen dan nam de vruchtbaarheid weer toe tot het normale peil. Ook bleek het mogelijk de ei-productie weer op het normale peil te brengen, wanneer deze door uithongering volledig stil was gelegd.

Onder meer uit het verschil in voedsel- en in ovipositievoorkeur voor aardappelloop en loof van donsnachtschade trokken wij de conclusie, dat de keuze van de waardplant als *voedsel* en als *ondergrond voor de afzetting van eieren* gedragingen zijn, die berusten op verschillende mechanismen. Dit verschil in voorkeur zal echter nauwelijks of geen consequenties hebben voor de overlevingskansen van de Coloradokever, daar deze na de verpoping eerst een rijpingsperiode doorbrengt op de plant van zijn voedselvoorkeur, waarop hij daarna zijn eieren zal leggen. De voedselkeuze bepaalt daardoor de plaats waar de eieren terecht zullen komen.

Hoewel het dus mogelijk is gebleken de waardplantkeuze van de Coloradokever te beïnvloeden, menen wij dat de waardplantbinding van *Leptinotarsa decemlineata* SAY geen gevolg kan zijn van een leer- of gewenningsproces.

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