AN EXPERIMENTAL INVESTIGATION OF THE INFLUENCE OF GLYCOSIDES, STEROIDS, AND OTHER PLANT CONSTITUENTS ON THE FEEDING BEHAVIOUR, DEVELOPMENT, AND SURVIVAL OF THE TWO-STRIPED GRASSHOPPER, *MELANOPLUS BIVITTATUS* (SAY), ACRIDIDAE; ORTHOPTERA

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ABSTRACT

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AN EXPERIMENTAL INVESTIGATION OF THE INFLUENCE OF GLYCOSIDES, STEROIDS, AND OTHER PLANT CONSTITUENTS ON THE FEEDING BEHAVIOUR, DEVELOPMENT, AND SURVIVAL OF THE TWO-STRIPED GRASSHOPPER, MELANOPLUS BIVITTATUS (SAY), ACRIDIDAE; ORTHOPTERA

The effects on survival, development, and feeding behaviour of Melanoplus bivittatus (Say) of twenty plant chemicals of possible significance in the determination of insect-host plant associations were investigated.

Effects on survival and development were studied in a series of growth experiments. Hatchlings were fed a chemically defined synthetic diet to which test chemicals were added.

Approximately half the test chemicals had no effect on survival and, with few exceptions, none affected adult weight or rate of weight gain.

Tigogenin was the only chemical which produced an unqualified increase in survival, although it did not affect feeding behaviour.

Nornicotine dipicrate, digitonin, solanine, tomatine, and saponin were lethal. However the last three chemicals did not inhibit feeding in preference experiments and were acceptable to the insect.

Approximately half the chemicals which inhibited feeding behaviour had only minor effects on survival and development. Hence the insect rejected some chemicals which were innocuous.

These data indicate that feeding behaviour of M. bivittatus is only partially correlated with its teleological needs.

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CHAPTER I

INTRODUCTION

The sometimes specific sometimes very broad range of plants fed upon by insects has been a subject of comment amongst men since very early times. During the last one and a half centuries entomologists have worked in the field of insect-host plant relationships, however it is only in recent years that any marked progress has occured. It is now recognised that insects respond characteristically to a wide range of plant chemicals and although various theories of host selection have been forthcoming all exponents agree that plant chemicals influence host selection by phytophagous insects.

The problem. The purpose of this study was to attempt to elucidate the effect of a number of 'secondary plant chemicals' (Fraenkel 1959) on the feeding behaviour, development, and survival of the two-striped grasshopper, Melanoplus bivittatus (Say). Secondary plant chemicals have been ascribed roles varying from that of attractant to that of repellent, some workers emphasising one role, some another. It was considered that further investigations using a range of chemicals related to the plants occuring within the geographic range of what has been generally considered a polyphagous insect would help in elucidating the role of such chemicals.

Importance of the study. At the present time a range of highly effective insecticides is available for the efficient control of many insect pests. However an ever increasing number of insects are developing resistance to more and more insecticides. Also the use of insecticides in various situations or at certain stages of growth of a crop is undesirable for such reasons as human health.

Hence it is increasingly important that all other possible avenues of insect control should be thoroughly explored. Not the least of these avenues is the development of insect resistant varieties of crop plants. Much success has been achieved in this field but to date the methods employed have lacked any general well authenticated basis. By enlarging our knowledge in the field of insect-host plant relationships the breeding of insect resistant plants can be developed beyond the present stage.

Also in the sphere of weed control many serious pests are not susceptible to herbicides or occur over such large areas or on land of such low value that biological control is the only economic means of control. The selection of insects to use against weeds also lacks any well authenticated basis. Greater knowledge in the insect-host plant field should enable research workers to better assess the potential value of insects being considered as biological control agents.

It is hoped that the data presented in this thesis will constitute a small advance in the field of insect-host plant relationships.

CHAPTER II

REVIEW OF THE LITERATURE

The literature dealing with the influence of plant constituents on insects is quite voluminous. Reviews on chemoreception have been presented by Dethier and Chadwick (1948), Dethier (1953, 1956), and Hodgson (1958, 1964). A review of literature pertaining to host selection in phytophagous insects was presented by Thorsteinson (1960a). The role of secondary plant substances in host selection has been stressed by Fraenkel (1959), and Fraenkel et al (1960). Here literature dealing with the influence of plant constituents on insects other than grasshoppers will be reviewed briefly and the literature dealing with the feeding behaviour of grasshoppers in more detail.

I. THE INFLUENCE OF PLANT CONSTITUENTS ON INSECTS OTHER THAN GRASSHOPPERS

Studies on the influence of plant constituents on host selection were pioneered by Verschaffelt (1910), who found a mustard oil glucoside to be an important factor in host selection by insects feeding on cruciferous plants. Later Thorsteinson (1953) found that the diamond-back moth, Plutella maculipennis (Curt.), an insect specific to the cruciferae, will feed on plants containing very low concentrations of mustard oil glucosides provided nutrients are present.

Nine glucosides isolated from tissues of cruciferous plants elicit feeding which increases with increasing concentration of an added nutrient, glucose (Nayar and Thorsteinson 1963). The influence of plant constituents on various other lepidopterous insects has been investigated. Dethier (1941, 1954) studied the larvae of Papilio species and concluded that essential oils or their constituents were important in the choice of host plants by these butterflies. Reactions of the European corn borer, Ostrinia = Pyrausta nubilalis (Hubn.) have been investigated by Beck (1956, 1957a, 1957b, 1957c, 1960), Beck, Kaske, and Smissman (1957), Beck and Hanec (1958), and Beck and Smissman (1960, 1961). Dietary sugars were found to have a pronounced positive effect on feeding behaviour; a resistance factor, 6-methoxybenzoxazolinone, was isolated from corn plant tissue; and a number of amino acids were found to increase the duration of larval feeding.

Yamamoto and Fraenkel (1959, 1960a, 1960b, 1960c) isolated an attractant and a gustatory stimulant for the tobacco hornworm, Protoparce sexta (Johan.) from host plants, and Waldbauer and Fraenkel (1961) and Waldbauer (1962) investigated feeding by maxillectomised larvae on normally rejected plants. Nayar and Fraenkel (1962) defined the chemical basis of host plant selection in the silkworm, Bombyx mori (L), and in the catalpa sphinx, Ceratonia catalpae (Boisduval) (Nayar and Fraenkel, 1963a). Hamamura (1959) and Hamamura et al (1961, 1962) working with the

silkworm, B. mori, have described attractants (citral, terpinyl acetate, linalyl acetate, linalol, and β-γ-haxanol), biting factors (β-sitosterol and isoquercitrin or morin), and as a swallowing factor cellulose plus sucrose, inositol, inorganic phosphate, and silica as co-factors. Also working with B. mori Ito et al (1964) have described the effects of sterols on feeding and nutrition.

Wensler (1962) concluded that sinigrin is a specific stimulus for host selection by the aphid, Brevicoryne brassicae (L), and is received via the stylets after they have penetrated the leaf surface. Brusse (1962) correlated high alkaloid content in a segregating population of Lupinus angustifolius L. with resistance to the aphid Macrosiphum euphorbiae (Thos.)

Within the Coleoptera responses of a number of species to constituents of their food plants have been investigated. Chauvin (1945) demonstrated that an extract of potato leaves stimulated feeding in the potato beetle, Leptinotarsa decemlineata Say. An attractant for the same species was isolated by Yamamoto and Fraenkel (1959). Byers et al (1961) found that extracts of bean seeds elicited feeding responses in the Mexican bean beetle, Epilachna varivestris Muls. The chemical basis of host selection in this species was further investigated by Nayar and Fraenkel (1963b). Leaf alcohol and some aliphatic alcohols were found to be attractants for the adult and larva of the vegetable weevil, Listroderes

costirostris obliquus Klug, (Matsumoto and Sugiyama, 1960). Working with the same species Matsumoto (1962) found coumarin to be both an olfactory attractant and a feeding inhibitor. In a series of papers (Jenkins et al 1963; Keller et al 1963; and Maxwell et al 1963a; 1963b) various extracts of cotton plants have been reported as arrestants, attractants, feeding stimulants, and repellents for the boll weevil, Anthomonus grandis Boh. Neff and Vanderzant (1963) have reported methods of evaluating the chemotrophic response of this species to extracts of the cotton plant and various other substances. Loschiavo et al (1963) demonstrated that benzene extracts of elm bark were arrestants and feeding stimulants for the smaller European elm bark beetle, Scolytus multistriatus Marsh. Loschiavo (1964) reviewed the literature dealing with the effect of food constituents on grain beetles and reported the responses of the confused flour beetle, Tribolium confusum Duval, to various substances isolated from grain and yeast.

The wide range in the influence of plant constituents on phytophagous insects is further emphasised by the investigations of Fennah (1960) on the water status of plants in relation to attack by sucking insects; Kushner and Harvey (1962) on the possible role of antibacterial substances in leaves in insect resistance to disease; Levinson (1962) on the role of dietary sterols; and Edelman and Efros (1962) on the effect of plant-growth stimulators.

II THE FEEDING BEHAVIOUR OF GRASSHOPPERS

An excellent review of literature dealing with feeding behaviour and nutrition in grasshoppers and locusts has been provided by Dadd (1963). Sighting work with various species, including Melanoplus spp. Dadd concludes (p.49) that "a tenable case exists for supposing that the food preferences which many polyphagous pest grasshoppers undoubtedly exhibit are partly the outcome of the discrimination of nutritionally advantageous features." As to how the acridids encounter their food plants, the literature indicates that olfactory stimuli generally operate only over small distances. Acridids have been observed to bite unsuitable food plants and other substrates before rejecting them. In such cases acceptibility may be determined as the first bite releases from the crushed tissue juices or odours which are possibly repellents or feeding inhibitors. Up to that point the tissue had been attractive or at least not repellent.

More attention has been devoted to the identification of feeding stimulants than to feeding inhibitants. Sugars, notably sucrose and glucose, and many other compounds likely to occur in plant tissues have been found to be effective feeding stimulants. Amongst the most notable are the phospholipids isolated from wheatgerm oil (Thorsteinson and Nayar 1963). A discovery critical to the development of

satisfactory synthetic diet was the need for inert bulk (cellulose) (Dadd 1960a).

A discussion of theories of food selection and a comprehensive review of nutrition in acridids is included in Dadd's review (Dadd 1963).

A number of workers have investigated the effect of various food plants upon growth and fecundity (Hodge 1933; Tauber et al 1945; Pfadt 1949; Smith and Northcotte 1951; Smith et al 1952; Smith 1958; Gangwere 1959; Pickford 1958, 1962; Putnam 1962; Barnes 1963; Mulkern et al 1964). In a monograph on food selection in Orthoptera Gangwere (1961) presented and critically appraised some excellent data on food selection in various species of Acrididae. The results of these investigations indicate wide divergence in the number and range of food plants eaten. In some species (eg. Melanoplus spp.) growth and fecundity differed widely when they were reared on different food plants. Some species exhibited a preference for those plants which supported maximum growth. However optimal growth was often recorded when the insects fed on more than one plant species. Melanoplus spp. seem to be in this category.

Dadd (1957, 1960a, 1960b, 1960c, 1960d, 1960e, 1961a, 1961b, 1961c) formulated a chemically defined synthetic diet and investigated the nutritional requirements of the locusts

Schistocerca and Locusta. This dry diet consisted of cellulose, sucrose, dextrin, a salt mixture, cholesterol, linoleic acid, casein, peptone, egg albumen, ascorbic acid, and ten water soluble vitamins of the B complex. It supported growth from hatching to the adult stage. Cavanagh (1963) investigated the use of this diet as a food for adult Schistocerca. It was found necessary to supply adults with a grass supplement in order to obtain egg clutches of normal size and fertility. Nayar (1964a, 1964b) developed a slightly simpler diet which supported growth of Camnula pelucida (Scudder) and Melanoplus bivittatus (Say) from hatching to the adult stage.

Very little has been published on the effects of glycosides and alkaloids on the feeding bahaviour of acridids. A report by Thorsteinson (1960b) indicated that barley plants sprayed with solutions of alkaloids were more resistant to grasshopper feeding. Tests in which discs of elder pith were treated with a variety of alkaloids gave similar results. However in these tests a few chemicals appeared to stimulate feeding. (Thorsteinson, unpublished data).

III THEORIES OF HOST PLANT SELECTION

Fraenkel, Dethier, Kennedy, and Thorsteinson have been the chief proponents of theories of host plant selection. A summary of Fraenkel's views appeared in a review by

Lipke and Fraenkel (1956) in which these authors discuss the question as to whether selection is governed (p32) "(a) by the nutritional superiority of the plant or region of the plant serving as food for the insect, or (b) by the presence or absence of attractants and repellents in plants of more or less uniform food value to which the parasitic species has become adapted." The attractants and repellents are termed token stimuli and the view is taken that they may act as trigger compounds which induce the uptake of true nutrients. Host preference is considered to begin (p.33) "when a given insect species, by genetic selection, overcomes the repellent effect of such a material, thereby gaining a new source of food and resulting ideally in a situation where further genetic selection produces strains of the original species who require the former repellent (now the attractant) to induce feeding." By and large these views are supported by Dethier (1953, 1954). However he tends to place greater emphasis on the role of token stimuli as repellents or feeding inhibitors, ie. on an early stage in the hypothetical evolutionary sequence proposed by Fraenkel.

Kennedy (1958) discusses the theories propounded by Fraenkel and Dethier in relation to a dual discrimination theory which he had proposed earlier (Kennedy and Booth 1951; and Kennedy 1953). (p.51) "This postulated that, in addition

to specific stimulatory substances of no nutritive value governing botanical preferences, universal substances such as amino acids which are of fundamental metabolic (nutritional) importance to plants and insects alike, also played a major part in aphid host relations." Assumptions basic to this theory were that the supply of these substances varied greatly and that they acted as immediate sensory stimuli for feeding and other responses either directly or through token stimuli physiologically associated with them. While willing to accept the view that token stimuli are often important in host selection he was unwilling to agree that nutrient substances are not important in this regard.

Thorsteinson (1960a) in a review of the literature pertaining to host selection in phytophagous insects discussed the previously mentioned theories and reiterated his own views on the subject. The point of view taken by Thorsteinson but perhaps not very explicitly stated is that good data are available for only a handful of species and that there is no good reason to claim that any particular category whether caloric or otherwise metabolically significant or not is 'more important' generally than any other. In particular species one might venture to make such comparisons but even here the metabolic requisites for survival will always be decisive and any individual

behavioural peculiarity must be compatible with these requisites. Whether such peculiarities have any survival value and, if so, what evolutionary and ecological interrelations may be constructed to account for them is an interesting field for conjecture but does not yet have a broad empirical base for a uniquely superior theory (Thorsteinson, personal communication). Thorsteinson's views on insect-host plant relationships bear a certain resemblance to those of Kennedy and lack the unfortunate bias inherent in the theories of Fraenkel and Dethier.

CHAPTER III

MATERIALS AND METHODS

The insect species used in these studies was the two-striped grasshopper, Melanoplus bivittatus (Say) (Acridoidea: Acrididae: Cyrtacanthacridinae). The species is described as a general feeder, preferring forbs. It is widespread throughout Canada and the United States except in southeastern United States (Brooks 1958). It is described as one of the four most important economic species. Outbreaks occur periodically and are responsible for crop losses estimated at many millions of dollars (Mitchener 1956).

For these experiments eggs were collected from natural oviposition sites within approximately 20 miles of Winnipeg, Manitoba. Eggs were collected in the fall and stored at a constant temperature of 4°C. until required. Diapause was broken and a high percentage hatching was obtained after approximately two months cold storage. Hatchlings were either used in tests or were reared in wooden cages similar to those developed for rearing Schistocerca (Hunter-Jones 1961). When reared in cages nymphs were fed a diet of lettuce and bran.

I. INFLUENCE OF TEST CHEMICALS ON DEVELOPMENT AND SURVIVAL

Growth experiments. In these experiments the effects of various chemicals of plant orign on development and survival of M. bivittatus were investigated. Chemicals to be used were selected following a literature study of the alkaloids, glycosides, and saponins recorded from plant species or genera believed to occur within the geographic range of M. bivittatus. A summary of this study is given in Appendix I. Other chemicals known to occur widely within the plant kingdom or of special interest for other reasons were also included. The test chemicals were added to a dry synthetic diet which was used to rear nymphs of M. bivittatus from hatching to the adult stage. The diet was a modification of that proposed by Nayar (1964a). Its composition is given in Table I. Diet was formulated as required, stored in airtight containers and held at -28°C. To facilitate comparison of results, test chemicals were usually added to the diet at equimolecular concentrations. By and large the levels approximated those found in plant tissues. However the latter is highly variable and it may be best to view the diets as a series of 'synthetic plants' or 'chemical models of plants' differing only in the secondary plant substance or test chemical added to each. The test chemicals and the concentration at which each was added to the diet are listed in Table II.

In growth experiments seven hatchlings were placed in a plastic petri dish 7cm diameter and 1.6cm deep. The lid was ventilated by an insert of fine plastic screen 4.3cm diameter. The bottom of the dish was lined with plastic screen. Diet in powder form was provided in plastic dishes 1.6cm diameter and 0.5cm deep. Water was provided by a wet cotton pad approximately 1.0cm diameter (Fig.1). Fresh diet and clean dishes were provided every two days, or as required. Water was supplied as required. Experiments were inspected daily. Nymphs were weighed every seven days and adults were weighed within 24 hr of moulting.

All experiments were conducted at a constant temperature of 31°C and a relative humidity of approximately 70 per cent. Constant illumination was provided by two 40 watt incandescent lamps (Fig.2).

In each experiment treatments were replicated ten times. Some treatments were included in more than one experiment.

Analysis of variance was performed on data on survival (number of individuals completing metamorphosis), adult weight (mg), and daily rate of weight gain over the period from hatching to moulting to adult (mg/day). For analysis figures for survival were transformed using the formula $\sqrt{x + 0.5}$. Values of least significant difference (L.S.D.)

were used to determine which results were different to results for control insects (Snedecor 1961).

Data regarding variations during pre-imaginal development, in survival of nymphs, in daily rate of weight gain, and in durations of stadia, were recorded for six test chemicals.

TABLE I

COMPOSITION OF DRY SYNTHETIC DIET

Cellulose fiber.....44g	
Sucrose.....10g	
Soluble starch.....10g	
Dextrose.....10g	
Salt mixture W..... 3g	
Casein.....20g	
Cholesterol.....0.4g	Dissolved in ethyl ether
Linoleic acid.....0.4g	
Lecithin.....1.0g	
Thiamine.....2.5mg	Dissolved in ammoniated ethanol
Riboflavin.....2.5mg	
Nicotinic acid.....5.0mg	
Pyridoxine.....2.5mg	
Folic acid.....2.5mg	
i-inositol.....30.0mg	
Calcium pantothenate...5.0mg	
p-aminobenzoic acid....2.5mg	
Choline chloride.....100.0mg	
Biotin.....0.1mg	
L-ascorbic acid.....0.4g	

TABLE II

CONCENTRATIONS AT WHICH TEST CHEMICALS WERE USED IN GROWTH AND FEEDING PREFERENCE EXPERIMENTS AND IN DRINKING RESPONSE TESTS

Test chemical	Concentration used in	
	Growth and preference experiments - % dry weight	Drinking tests
Gramine	0.37	Saturated
Hordenine sulphate	1.00	(0.10M (0.02M (0.005M
Hydrastine hydrochloride	0.91	0.10M
Hyoscyamine hydrochloride	0.72	0.10M
Lobeline sulphate	1.67	0.10M
Lupinine	0.37	0.10M
Nornicotine dipicrate	1.33	-
Santonine	0.54	0.10M
Solanine	1.89	-
Tomatine	2.24	-
Veratrine	1.00	5.0%
Arbutin	0.59	(Saturated (0.10M
Indican	0.65	0.10M
Digitonin	(3.00 (1.00	(0.10M (0.02M
Diosgenin	1.00	-
Hecogenin	1.00	-
Saponin	1.00	(10.0% (0.5%
Tigogenin	1.00	-
β-sitosterol	0.91	4.15%
Stigmasterol	0.89	-

- indicates drinking tests were not conducted.

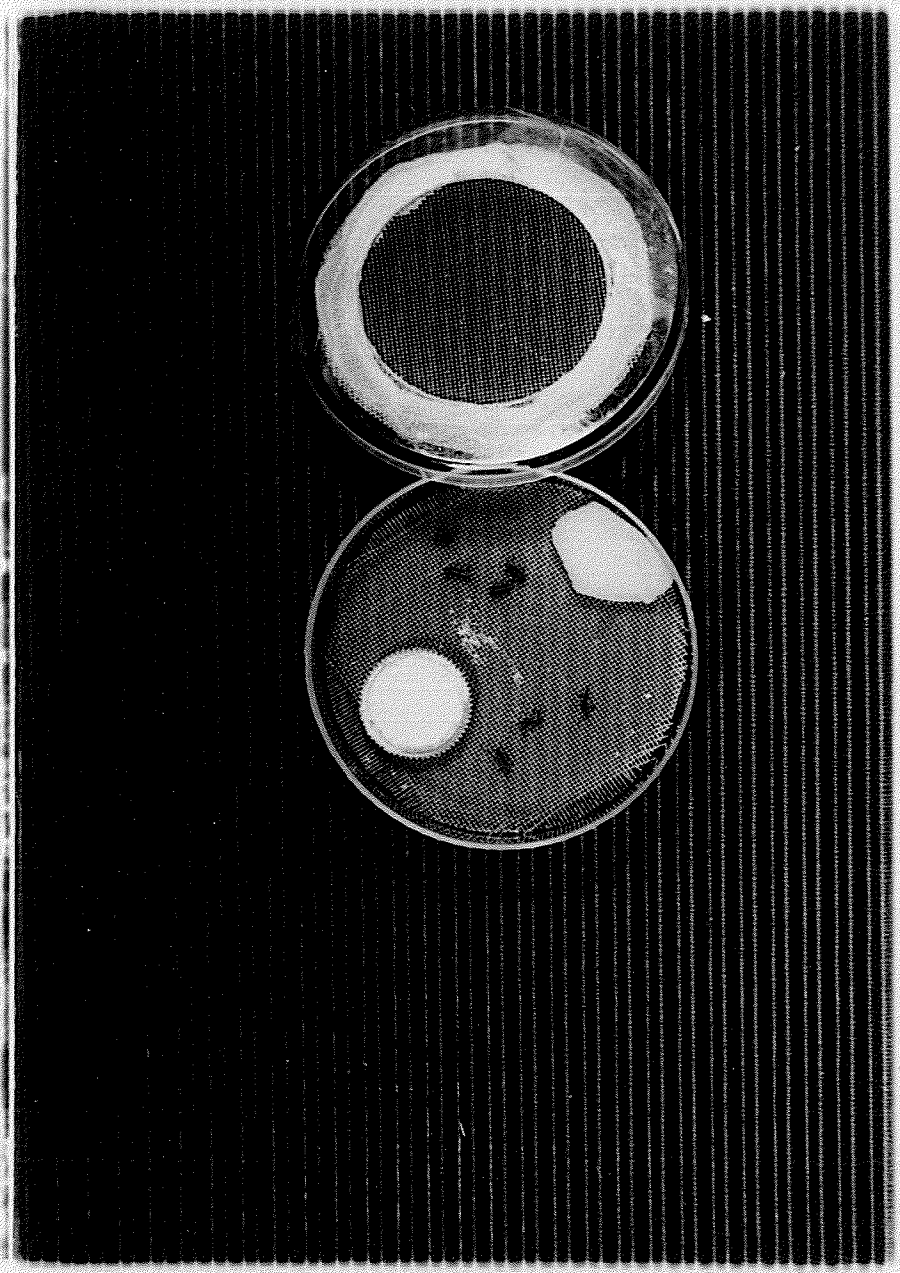

Fig. 1.- Petri dish containing diet, wet cotton pad, screen insert, and seven hatchlings as used for the feeding experiments.

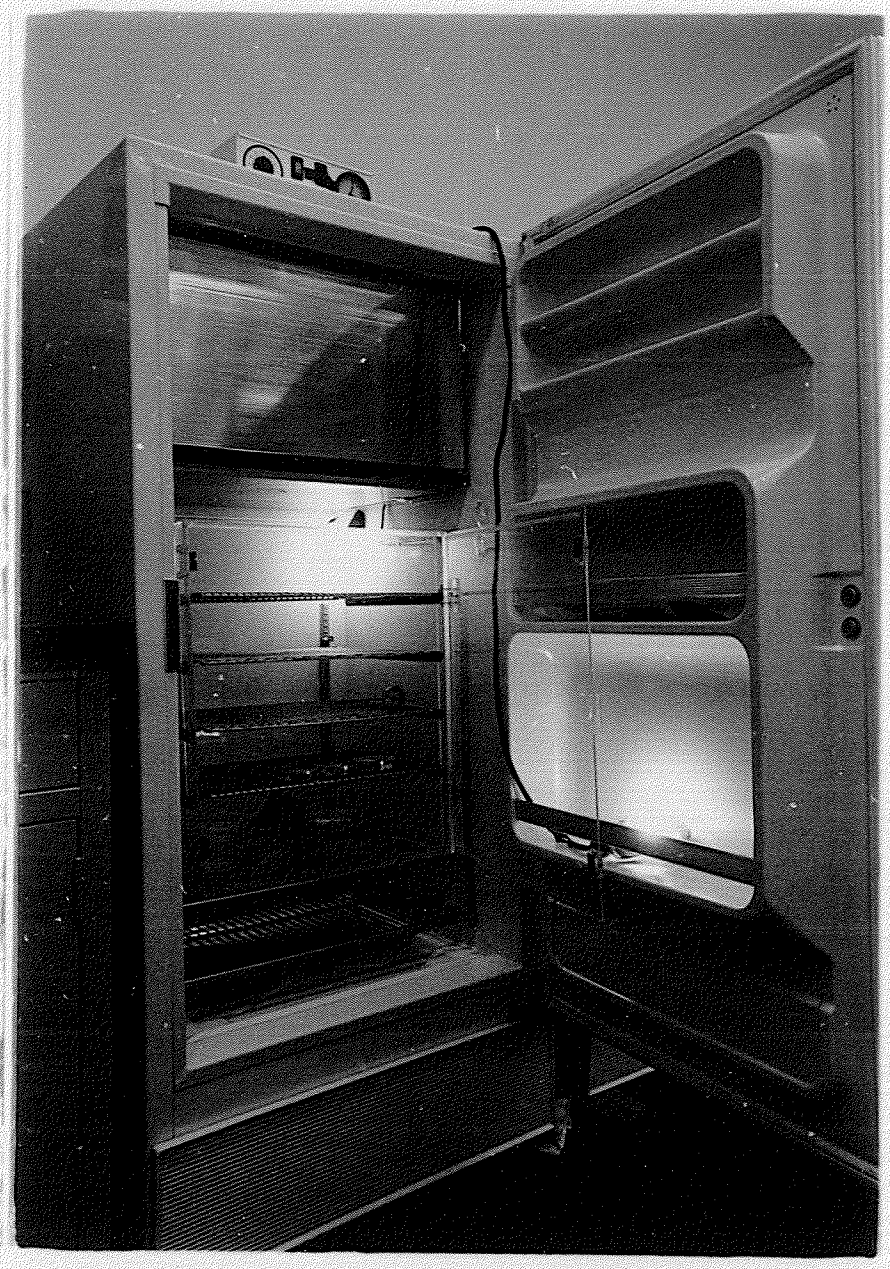

Fig. 2.- Incubator used for growth and preference experiments.

II. INFLUENCE OF TEST CHEMICALS ON FEEDING BEHAVIOUR

(a) Inhibition of drinking response. Responses to some of the test chemicals were investigated by applying aqueous solutions or emulsions to the distal segments of the maxillary and labial palps of adult M. bivittatus. Tests of this type have been used by various workers (for example, Frings and Frings 1956a, 1956b; Feir et al 1961) to determine behavioural responses of insects to chemicals. Here the method was employed to give an indication whether test chemicals used in growth experiments influenced the behaviour of M. bivittatus.

For testing, grasshoppers were confined in pieces of glass tubing supported in blocks of modelling clay. Water and solutions were applied to the palps with fine wire loops affixed to applicator sticks (Fig.3). Solutions were normally used at a concentration of one-tenth molar. Emulsions were used at an equivalent concentration (Table II).

Prior to testing grasshoppers were held for three to five hours without food or water. Only those individuals from which water elicited a 'drinking response' were used for testing. Drinking response may be defined as mandibular action associated with an attempt to drink. It may be induced by applying water to the distal segments of the maxillary and labial palps. The basis of the

tests was the inhibition of this response by test chemicals. Male grasshoppers were found to give more consistent results than females and were selected for most tests.

In the tests a grasshopper was tested in rapid succession with water, test chemical, and water. The sequence is summarised in Table III. Each test was replicated at least ten times.

Data on the inhibition of drinking response were arranged in a quartile distributin.

(b) Feeding preference experiments. The influence of test chemicals on the behaviour of M. bivittatus was further studied in a series of feeding preference experiments. In these experiments fifth-instar nymphs were provided with two food sources, each source being in the form of a 'wafer' of synthetic diet. A test chemical was added to one of these diets at the same level used in the feeding experiments. No test chemical was added to the other diet, which served for control. Wafers (2.8cm diameter and 0.3cm thick) were formed from powdered diet by compressing a standard volume at 2,000 lb/sq.in. with an hydraulic press.

Nymphs were reared on a diet of lettuce and bran and were used for these experiments within 24 hrs of moulting to the fifth instar. Three nymphs were

placed in plastic dishes such as were used in the feeding experiments and provided with two wafers, one of which contained a test chemical. Water was provided by cotton pads. The wafers were tilted to facilitate feeding (Fig. 4). Portions of wafers which remained uneaten after three days were photographed and their volumes were measured. Volume was measured as tenth-ml of water displaced. To prevent wafers disintegrating when placed in water the uneaten portions were waterproofed with a celloidin solution. The displacement apparatus is shown in Figure 5.

Data were analysed using Student's t-test (Snedecor 1961).

TABLE III

ROUTINE SEQUENCE FOR TESTING THE RESPONSES OF *M. BIVITTATUS* TO STIMULATION OF SENSORY RECEPTORS ON THE MAXILLARY AND LABIAL PALPS WITH SOLUTIONS OR EMULSIONS OF CHEMICALS

SEQUENCE	SITE	SUBSTANCE APPLIED
1	Right hand palps	Water
2	Right hand palps	Chemical
3	Right hand palps	Water
4	Left hand palps	Water

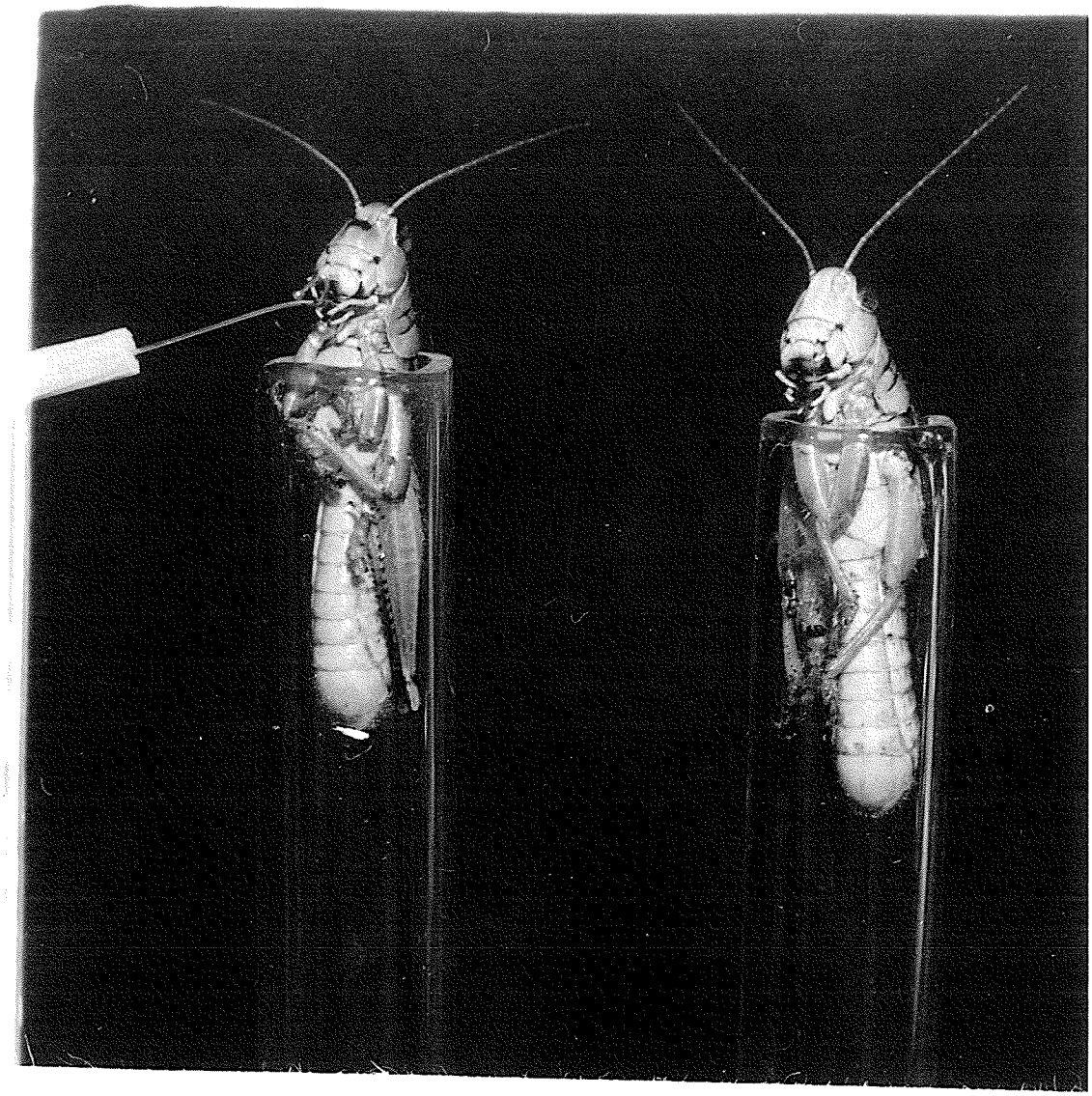

Fig. 3.- Adult grasshoppers held for drinking tests. The method of presentation of water and chemicals is illustrated with the specimen on the left.

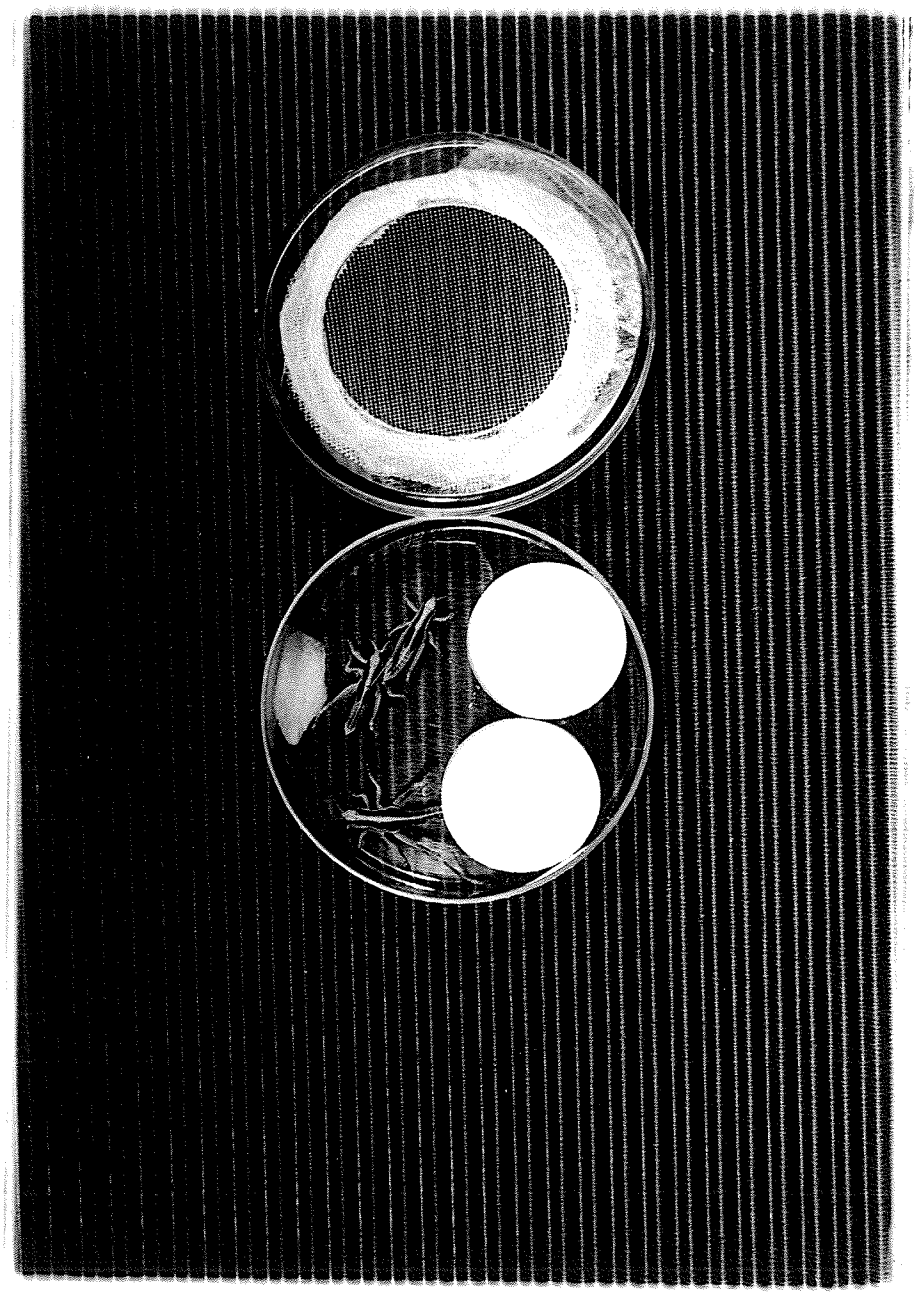

Fig. 4.- Petri dish containing a treatment and a control wafer, wet cotton pad, and three fifth instar nymphs as used for the preference experiments.

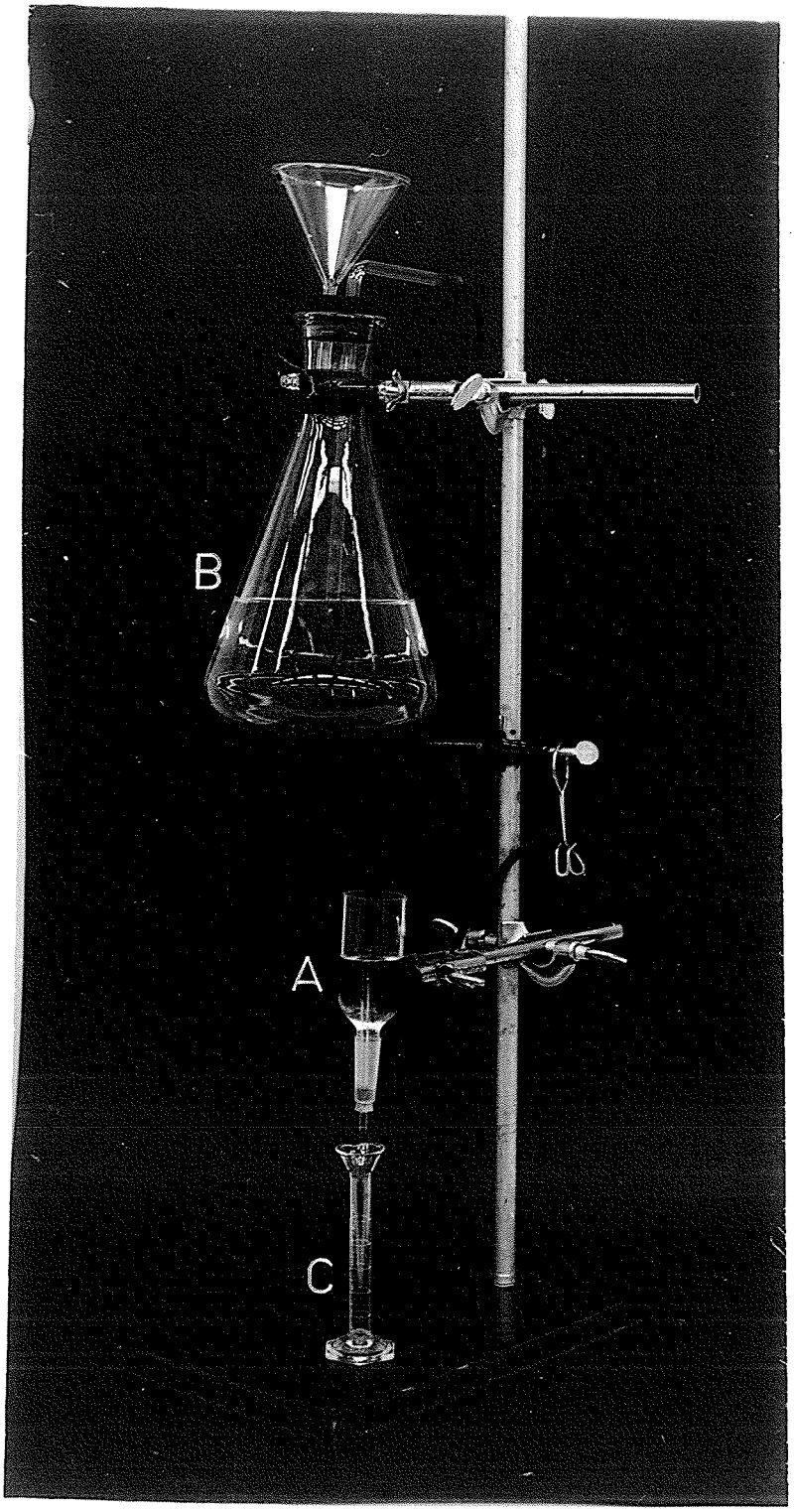

Fig. 5.- Apparatus used for measuring the volume of portions of wafers not eaten in preference experiments. Displacement cylinder, A, was filled to a constant level from reservoir, B. The volume of water displaced from A by a wafer was measured in the graduated cylinder, C.

CHAPTER IV

RESULTS AND DISCUSSION

I. INFLUENCE OF TEST CHEMICALS ON DEVELOPMENT AND SURVIVAL

(a) Growth experiments.

(i) Results. Results of individual growth experiments are presented in Tables IV to XIII, and are summarised in Table XIV and Figure 6. Data on survival of nymphs are presented in Figures 7 to 12, on durations of stadia in Figures 13 to 18, and on mean daily rates of weight gain in Figures 19 to 30.

Hatchlings were observed feeding on diets containing each test chemical and faeces were always found. Hence, deaths of nymphs may be attributed to metabolic effects of the chemicals and not to starvation due to inhibition of feeding.

To facilitate discussion, the test chemicals have been divided into two groups. One group contains ten compounds with steroid configuration, and the other group ten non-steroidal compounds.

The group of steroids includes three alkaloids, solanine, tomatine, and veratrine. Solanine and tomatine occur in plants of a notoriously toxic family, the Solanaceae. Solanine has been reported as hemolytic, and

tomatine is stated to precipitate cholesterol. A salt of solanine has insecticidal properties. (Webb 1948; Henry 1949; Stecher 1960). Addition of either of these chemicals to the diet of grasshopper hatchlings resulted in all dying before becoming adult (Tables XI, XIV, Fig. 6).

Veratrine significantly reduced the mean daily rate of weight gain but did not affect mean survival or mean adult weight (Tables XII, XIV, Figs. 6). The preparation used was a commercial product which contained the alkaloids cevadine, veratridine, cevadilline, sabadine and cevine. It is described as poisonous, exceedingly irritating to mucous membranes and when ingested by man may cause violent vomiting, purging, and intense burning of the mouth and throat (Stecher 1960).

The saponins and sapogenins, digitonin, diosgenin, hecogenin, saponin, and tigogenin, all have a steroid configuration. Digitonin was fed at one per cent and three per cent, dry weight. At one per cent it had no significant effect on mean survival or mean daily rate of weight gain, but significantly reduced mean adult weight. When the diet contained three per cent, all nymphs died before becoming adult. Working with Musca vicina Macq., Levinson and Bergmann (1957) reported that addition of digitonin to the diet of larvae reduced growth and survival with increasing concentration, higher concentrations

(0.10 per cent and 0.17 per cent) being lethal. They postulated that these effects were due to the ability of digitonin to precipitate steroids possessing a 3 β-hydroxyl group.

Diosgenin significantly reduced mean survival but had no significant effect on mean adult weight or mean daily rate of weight gain (Tables XII, XIII, XIV, Fig. 6).

Hecogenin was included in two growth experiments. In one, mean survival was reduced but mean daily rate of weight gain was not significantly different to that of control insects. In the other, both survival and mean daily rate of weight gain were increased. However in both experiments mean adult weight was not significantly different to that for control insects (Tables XII, XIII, XIV Fig. 6).

The addition of saponin to the diet resulted in the death of all nymphs (Tables VII, VIII, XIV, Fig. 6). Saponin added to the diet of larvae of Musca vicina was lethal (Levinson and Bergmann 1957). The leaves and bark of plants containing saponin have been used as fish poisons by primitive races since early times. Saponins have little effect when ingested by higher vertebrates but are strongly hemolytic if they enter the bloodstream (Stecher 1960). The saponin used in these experiments was a commercial preparation made from the bark of Quillaia salonaria and contained quillaic acid and sapotoxin (Rosen, K.B., Mann

Research Laboratories Inc., personal communication).

Tigogenin was included in two growth experiments. In both experiments mean survival was increased significantly, but mean adult weight was not significantly different to that for control insects. In one experiment mean daily rate of weight gain was increased, in the other it was not significantly different (Tables IX, XIII, XIV, Fig. 6). In the experiment in which rate of weight gain was increased, mortality was highest during the first instar and nearly consistent during the second, third, fourth, and fifth instars. The pattern was similar to that for control insects (Fig. 7). As development proceeded beyond the first instar, stadia became increasingly shorter than for control insects (Fig. 13). Throughout development the mean daily rate of weight gain increased continuously and was higher than for control insects. The greatest increase in daily rate of weight gain occurred during the period fourteen to twenty-one days after hatching (Figs. 19, 25). With the possible exception of hecogenin, tigogenin was the only test chemical which under the conditions of these experiments, increased survival and rate of development, and appeared to have a stimulating effect on nymphs of M. bivittatus. However in spite of these effects mean adult weight did not differ significantly from that of control insects.

The sterols, β-sitosterol and stigmasterol, produced no significant effects on survival or development (Tables XI, XIV, Fig. 6).

The group of non-steroid test chemicals includes eight alkaloids, gramine, hordenine sulphate, hydrastine hydrochloride, lobeline sulphate, lupinine, nornicotine dipicrate, and santonine. Gramine did not significantly affect mean survival or mean daily rate of weight gain, but reduced mean adult weight (Tables IX, XIV, Fig. 6).

Hordenine sulphate was included in three growth experiments. It had no significant effect on survival or overall development. (Tables IV, VI, XIV, Fig. 6). However in comparison with control insects mortality was lower in the first, second, and third instars, and higher in the fourth and fifth instars (Fig. 8). Stadia were of similar duration to those of control insects (Fig. 14). The mean daily rate of weight gain was similar to that of control insects (Figs. 20, 26). Hence the only effect which hordenine sulphate appeared to have in these experiments was a reduction in mortality in early instars which was balanced by an increase in mortality in later instars.

Hydrastine hydrochloride had no significant effect on mean survival or mean adult weight but reduced the mean daily rate of weight gain (Tables V, XIV, Fig. 6). This compound is reported to have a strychnine-like action on higher animals (Stecher 1960).

Hyoscyamine hydrochloride had no significant effect on mean survival or mean adult weight (Tables IV, XIV, Fig.6). Mortality followed a pattern similar to that for control insects (Fig. 9). Mean daily rate of weight gain (Figs. 21, 27) and durations of stadia (Fig. 15) were also similar to those of control insects.

Lobeline sulphate had no significant effect on mean survival or mean adult weight (Tables V, XIV, Fig. 6). Survival followed a pattern similar to that for control insects (Fig. 11). The mean daily rate of weight gain was less than for control insects throughout the pre-imaginal period (Fig. 23), and the difference in these rates increased as development proceeded (Fig. 29). Durations of stadia are shown in Figure 17.

Lupinine was included in three experiments. In one, mean survival, mean adult weight, and mean daily rate of weight gain were not significantly affected (Tables X, XIV, Fig. 6). In another experiment, mean survival, mean adult weight, and mean daily rate of weight gain were reduced significantly (Tables IX, XIV, Fig. 6). In a third experiment, mean survival and mean daily rate of weight gain were reduced significantly while mean adult weight was not affected (Tables XII, XIV, Fig. 6).

Nornicotine has been recorded as having insecticidal properties and as being a more efficient contact poison

than the well known insect poison, nicotine (Stecher 1960). In growth experiments with nornicotine dipicrate all nymphs died before becoming adult (Tables VII, VIII, XIV, Fig. 6). However a number of insects (for example, Protoparce sexta Johan.) feed on plants containing quite high concentrations of nicotine and apparently have some mechanism whereby they can excrete or detoxify this chemical (Self et al 1964).

Santonine was included in two growth experiments. In one, mean survival was not significantly affected while in the other it was reduced. In both experiments mean adult weight and mean daily rate of weight gain were not significantly different to those for control insects.

The non-steroidal group of compounds included the glucosides, arbutin and indican. Addition of arbutin to the diet of hatchlings had no significant effect on mean survival or mean adult weight (Tables IV, XIV, Fig. 6). Mortality occurred at approximately the same rate in all instars and followed a pattern similar to that for control insects (Fig. 10). The mean daily rate of weight gain was not significantly different to that of control insects and showed no marked fluctuations. It increased with age, and followed a pattern similar to that for control insects (Figs. 22, 28). After the first instar, durations of stadia increased with age and were similar to those of control insects (Fig. 16). It may be concluded that under the

conditions of the experiment arbutin had no significant effect on survival or development of M. bivittatus.

Indican significantly reduced mean survival but did not affect mean adult weight or mean rate of weight gain. Mortality during the first instar was greater than for control insects (Fig. 12). The mean daily rate of weight gain was higher than for control insects from hatching to fourteen days after hatching but was lower from fourteen to twenty-one days after hatching (Fig. 30). The duration of each stadium was consistently shorter than corresponding stadia for control insects (Fig. 18).

(ii) Discussion. Addition of various steroid chemicals, (solanine, tomatine, veratrine, digitonin, diosigin, saponin, and possibly hecogenin) to a synthetic diet which contained cholesterol at a level adequate for normal growth, adversely affected survival or development, or was lethal to nymphs of M. bivittatus. The sterols β-sitosterol and stigmasterol did not affect the insect. Tigogenin, and possibly hecogenin increased survival and rate of development of the insect (Table XIV, Fig. 6).

All insect species which have been investigated have been unable to synthesise their sterol requirements, and a dietary source of a suitable sterol is essential. Cholesterol has satisfied the sterol requirements of all insects

investigated (Gilmour 1961; Clayton 1964). However, cholesterol has been found in only a few plants (Johnson et al 1963). Phytosterols (for example, β-sitosterol and stigmasterol) can supply the needs of phytophagous insects and in some species appear to be utilized more effectively than cholesterol (Levinson 1962; Clayton 1964). Reporting investigations on the feeding behaviour of Bombyx mori (L.), Ito et al (1964) have classified these sterols as feeding stimulants, and Hamamura and his associates (1961, 1962) have termed β-sitosterol a 'biting factor'. Nayar and Fraenkel (1962) found the chemical purity of β-sitosterol to influence behaviour of B. mori.

It has been suggested that insects utilize sterol as a structural component of cells, as an anti-infective agent (Levinson 1962), and as a precursor of steroid hormones (Clayton 1964). In this regard it is interesting to note that diosgenin, tigogenin, and stigmasterol are starting materials or intermediates in the synthesis of steroid hormones (Fieser and Fieser 1959). Whether this common property is of significance in the effects of these compounds on insects is not known. Some insects have been reared using suboptimal quantities of cholesterol provided another ('sparing') sterol is contained in the diet. It has been suggested that the cholesterol is utilized in hormone metabolism and fulfills other highly specific roles, and the sparing sterol fulfills roles of lower specificity

(Clayton 1964). Several workers have reported that certain analogues of cholesterol inhibit insect growth. However further confirmatory work is required (Clayton 1964; Levinson and Bergmann 1957).

These findings, together with those cited in discussion of specific chemicals used in these experiments with M. bivittatus, indicate that ingestion by insects of certain steroid chemicals may interfere with metabolism and utilization of sterols essential to the insect. Interference with the sterol metabolism would seriously influence survival and development and may be lethal. However other steroid chemicals appear to have a sparing action on, or may themselves completely supply, the sterol requirements of the insect. The findings reported here for M. bivittatus are in general agreement with those reported by various workers for other insects.

A striking feature of these growth experiment data was the relative uniformity of mean adult weight regardless of diet. For seventeen test chemicals mean adult weights were not significantly different to those of control insects. Nymphs tended to grow to uniform adult weight regardless of whether their diet contained a chemical which increased survival and rate of weight gain (for example, tigogenin), one which had no effect on survival and rate of weight gain (for example, hordenine sulphate, hyoscyamine

hydrochloride, or arbutin), one which had no effect on survival but decreased rate of weight gain (for example, lobeline sulphate), or one which decreased survival and decreased rate of weight gain (for example, indican).

However, mean daily rate of weight gain was influenced by tigogenin, hydrastine hydrochloride, lobeline sulphate, veratrine, and possibly hecogenin, all of which did not affect adult weight.

In the attainment of a given weight, rate of weight gain and duration of development period are necessarily related. Hence tigogenin, and possibly hecogenin, which increased daily rate of weight gain decreased the duration of the pre-imaginal period, and hydrastine hydrochloride, lobeline sulphate, and veratrine, which decreased daily rate of weight gain increased the duration of the pre-imaginal period.

TABLE IV

RESULTS OF FEEDING HATCHLINGS DIETS CONTAINING ARBUTIN, HYOSCYAMINE HYDROCHLORIDE, OR HORDENINE SULPHATE

Chemical added to basic diet	Mean number per replicate completing metamorphosis		Mean adult weight - mg	Mean rate of weight gain mg/day
	Actual	Transformed		
Arbutin	2.6	1.721	265.2	7.53
Hyoscyamine hydrochloride	3.0	1.796	281.0	8.06
Hordenine sulphate	2.3	1.638	276.7	7.73
Control (no test chemical)	2.7	1.764	288.8	7.86
F value		0.033	0.38	0.28

Total d.f. = 39

$F_{0.05}$ = 2.96

TABLE V

RESULTS OF FEEDING HATCHLINGS DIETS CONTAINING HORDENINE SULPHATE, HYDRASTINE HYDROCHLORIDE, OR LOBELINE SULPHATE

Chemical added to basic diet	Mean number per replicate completing metamorphosis		Mean adult weight - mg	Mean rate of weight gain mg/day
	Actual	Transformed		
Hordenine sulphate	2.8	1.767	308.9	12.38
Hydrastine hydrochloride	2.8	1.800	303.3	11.37*
Lobeline sulphate	2.9	1.837	297.4	10.22*
Control (no test chemical)	2.6	1.732	328.6	13.62
F value		0.024	0.68	4.45
$L.S.D._{0.05}$				0.971

Total d.f. = 39 $F_{0.05} = 2.96$ $F_{0.01} = 4.60$

* significantly different to control at 0.05

TABLE VI

RESULTS OF FEEDING HATCHLINGS DIETS CONTAINING SANTONINE, INDICAN, OR HORDENINE SULPHATE

Chemical added to basic diet	Mean number per replicate completing metamorphosis		Mean adult weight - mg	Mean rate of weight gain mg/day
	Actual	Transformed		
Santonine	0.5	0.95*	396.0	15.3
Indican	0.7	1.04*	341.0	15.3
Hordenine sulphate	1.3	1.29	325.9	13.2
Control (no test chemical)	1.6	1.41	359.7	14.0
F value		4.06	2.73	3.48
$L.S.D._{0.05}$		0.312		1.64

Total d.f. = 39 $F_{0.05}$ = 2.96 $F_{0.01}$ = 4.60

* significantly different to control at 0.05

TABLE VII

RESULTS OF FEEDING HATCHLINGS DIETS CONTAINING SANTONINE, NORNICOTINE DIPICRATE, OR SAPONIN

Chemical added to basic diet	Mean number per replicate completing metamorphosis		Mean adult weight - mg	Mean rate of weight gain mg/day
	Actual	Transformed		
Santonine	2.2	1.6	319.5	11.86
Nornicotine dipicrate	0.0	0.7**	-	-
Saponin	0.0	0.7**	-	-
Control (no test chemical)	2.3	1.6	340.1	12.37
F value		30.5	1.36	0.14
$L.S.D._{0.05}$		0.271		
$L.S.D._{0.01}$		0.366		

Total d.f. = 39 (19) $F_{0.05}$ = 2.96 (5.12) $F_{0.01}$ = 4.60

Figures in parenthesis refer to data in columns 4 and 5.

** significantly different to control at 0.01

TABLE VIII

RESULTS OF FEEDING HATCHLINGS DIETS CONTAINING SAPONIN OR NORNICOTINE DIPICRATE

Chemical added to basic diet	Mean number per replicate completing metamorphosis		Mean adult weight - mg	Mean rate of weight gain mg/day
	Actual	Transformed		
Saponin	0.0	0.71**	-	-
Saponin	0.0	0.71**	-	-
Nornicotine dipicrate	0.0	0.71**	-	-
Nornicotine dipicrate	0.0	0.71**	-	-
Control (no test chemical)	1.7	1.47	360.5	15.34
F value		127.6		
$L.S.D._{0.01}$		0.109		

Total d. f. = 49 $\quad F_{0.01} = 3.89$

** significantly different to control at 0.01

TABLE IX

RESULTS OF FEEDING HATCHLINGS DIETS CONTAINING GRAMINE, LUPININE, DIGITONIN, OR TIGOGENIN

Chemical added to basic diet	Mean number per replicate completing metamorphosis		Mean adult weight - mg	Mean rate of weight gain mg/day
	Actual	Transformed		
Gramine	0.7	1.037	289.9*	9.69
Lupinine	0.1	0.761*	229.0 **	7.40*
Digitonin (1%)	0.8	1.081	244.1*	8.10
Tigogenin	1.6	1.388*	374.1	13.07**
Control (no test chemical)	0.8	1.066	364.0	9.86
F value		3.44	11.93	10.11
$L.S.D._{0.05}$		0.035	55.57	1.98
$L.S.D._{0.01}$			74.58	2.66

Total d.f. = 49 $F_{0.05}$ = 2.63 $F_{0.01}$ = 3.89

* significantly different to control at 0.05

** significantly different to control at 0.01

TABLE X

RESULTS OF FEEDING HATCHLINGS DIETS CONTAINING LUPININE

Chemical added to basic diet	Mean number per replicate completing metamorphosis		Mean adult weight - mg	Mean rate of weight gain mg/day
	Actual	Transformed		
Lupinine	2.2	1.60	290.4	7.99
Control (no test chemical)	2.3	1.64	286.1	9.31
F value		0.005	0.004	2.73

Total d.f. = 19

TABLE XI

RESULTS OF FEEDING HATCHLINGS DIETS CONTAINING SOLANINE, TOMATINE, STIGMASTEROL, OR β-SITOSTEROL

Chemical added to basic diet	Mean number per replicate completing metamorphosis		Mean adult weight - mg	Mean rate of weight gain mg/day
	Actual	Transformed		
Solanine	0.0	0.71**	-	-
Solanine	0.0	0.71**	-	-
Tomatine	0.0	0.71**	-	-
Stigmasterol	2.4	1.69	316.2	9.36
β-sitosterol	2.5	1.81	333.9	10.36
Control (no test chemical)	2.3	1.65	346.6	10.81
F value		72.5	0.72	1.96
$L.S.D._{0.05}$		0.19		
$L.S.D._{0.01}$		0.25		

Total d.f. = 59 (29) $F_{0.05}$ = 2.43 (3.35) $F_{0.01}$ = 3.46 (6.01)

Figures in parenthesis refer to data in columns 4 and 5.

** significantly different to control at 0.01

TABLE XII

RESULTS OF FEEDING HATCHLINGS DIETS CONTAINING

DIOSGENIN, HECOGENIN, VERATRINE, OR LUPININE

Chemical added to basic diet	Mean number per replicate completing metamorphosis		Mean adult weight - mg	Mean rate of weight gain mg/day
	Actual	Transformed		
Diosgenin	0.8	1.088**	328.8	14.27
Hecogenin	0.9	1.109**	319.9	12.38
Veratrine	1,7	1.355	307.7	11.37*
Lupinine	0.5	0.950**	371.9	11.59*
Control (no test chemical)	1.4	1.316	351.6	14.22
F value		16.82	1.54	3.21
$L.S.D._{0.05}$		0.118		
$L.S.D._{0.01}$		0.158		

Total d.f. = 49 $F_{0.05}$ = 2.63 $F_{0.01}$ = 3.89

* significantly different to control at 0.05

** significantly different to control at 0.01

TABLE XIII

RESULTS OF FEEDING HATCHLINGS ON DIETS CONTAINING DIOSGENIN, HECOGENIN, TIGOGENIN, OR DIGITONIN

Chemical added to basic diet	Mean number per replicate completing metamorphosis		Mean adult weight - mg	Mean rate of weight gain mg/day
	Actual	Transformed		
Diosgenin	0.6	1.001**	336.2	12.70
Hecogenin	2.8	1.618*	339.0	14.19*
Tigogenin	2.6	1.666**	316.2	12.49
Digitonin (3%)	0.0	0.71 **	-	-
Control (no test chemical)	2.0	1.580	327.3	11.25
F value		11.09	0.396	3.93
$L.S.D._{0.05}$		0.037		1.76
$L.S.D._{0.01}$		0.050		

Total d.f. = 49 (39) $F_{0.05}$ = 2.63 (2.96) $F_{0.01}$ = 3.89 (4.60)

Figures in parenthesis refer to data in columns 4 and 5.

* significantly different to control at 0.05

** significantly different to control at 0.01

TABLE XIV

RESULTS OF GROWTH EXPERIMENTS IN WHICH NYMPHS WERE FED DIETS TO WHICH TEST CHEMICALS WERE ADDED

Test chemical	Survival			Adult weight			Rate of weight gain		
	T > C	T = C	T < C	T > C	T = C	T < C	T > C	T = C	T < C
Gramine		1			1			1	
Hordenine sulphate		1,2,3			1,2,3			1,2,3	
Hydrastine hydrochloride		1			1				1
Hyoscyamine hydrochloride		1			1			1	
Lobeline sulphate		1			1				1
Lupinine		1	2,3		1,3	2		1	2,3
Nornicotine dipicrate*			1,2,3						
Santonine		1	2		1,2			1,2	
Solanine*			1,2						
Tomatine*			1						
Veratrine		1			1				1
Arbutin		1			1			1	
Indican			1		1			1	
Digitonin (1%)		1				1		1	
(3%)*			1						
Diosgenin			1,2		1,2			1,2	
Hecogenin	1		2		1,2		1	2	
Saponin*			1,2,3						
Tigogenin	1,2				1,2		1	2	
-sitosterol		1			1			1	
Stigmasterol		1			1			1	

For any chemical, 1 indicates results from one experiment, 2 results from a second experiment, and 3 results from a third experiment.

* chemicals which were lethal.

T = diet to which test chemical was added.

C = control diet.

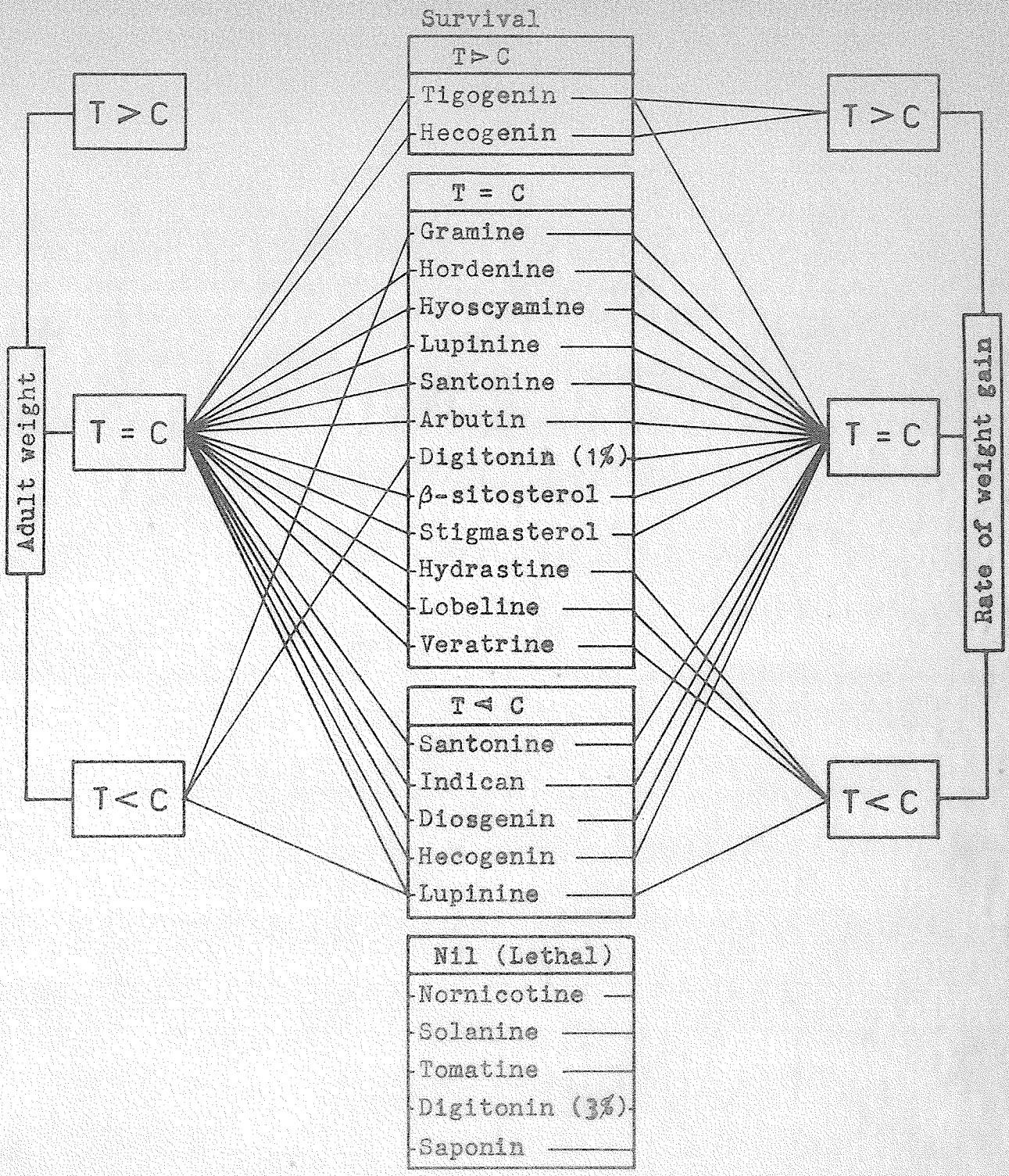

Fig. 6.- Results of growth experiments in which hatchlings were fed diets containing test chemicals.

T = diet to which test chemical was added.

C = control diet.

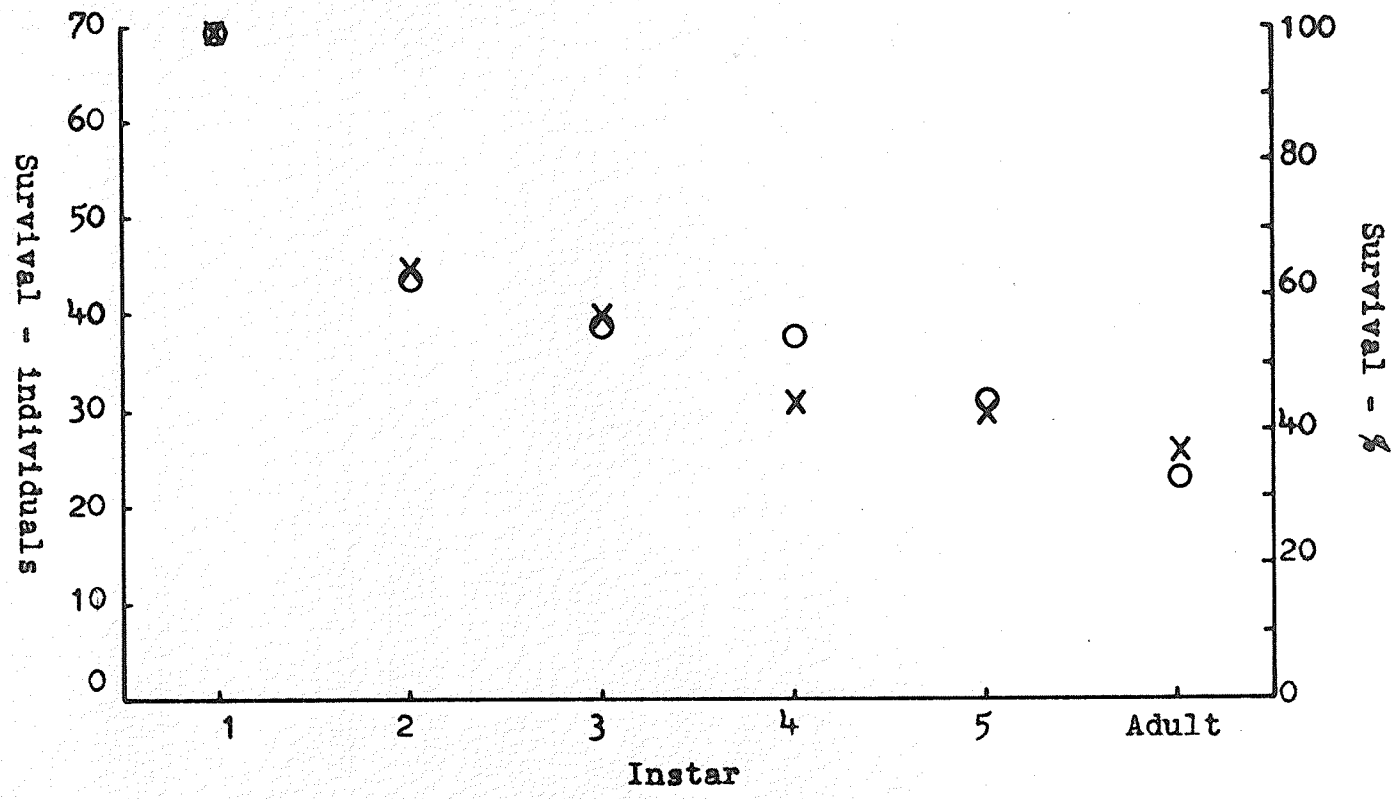

Fig. 7.- Survival, by instars, of nymphs fed diet containing 1.0% tigogenin. This treatment significantly increased the number becoming adult.

X Treatment O Control

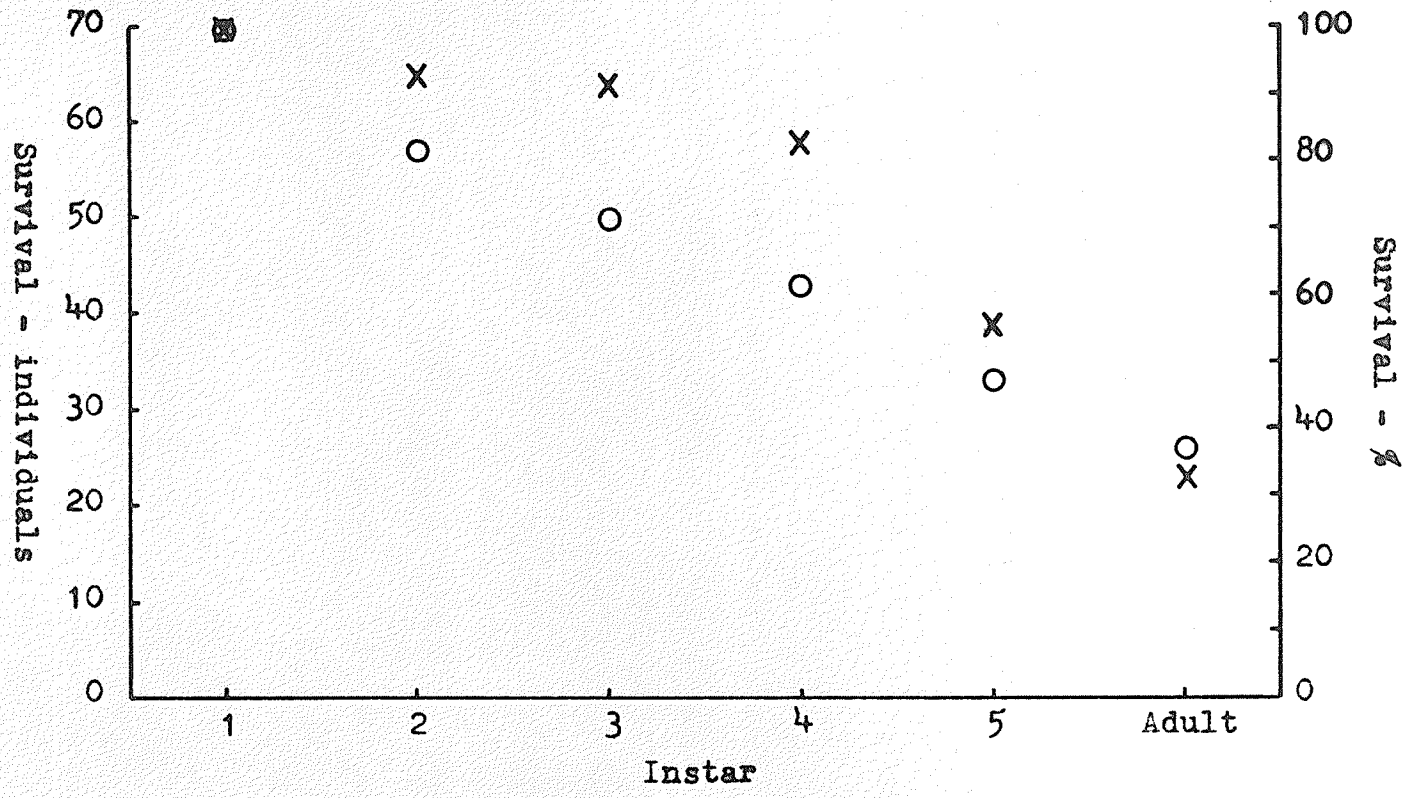

Fig. 8.- Survival, by instars, of nymphs fed diet containing 1.0% hordenine sulphate. This treatment did not significantly affect the number becoming adult.

X Treatment O Control

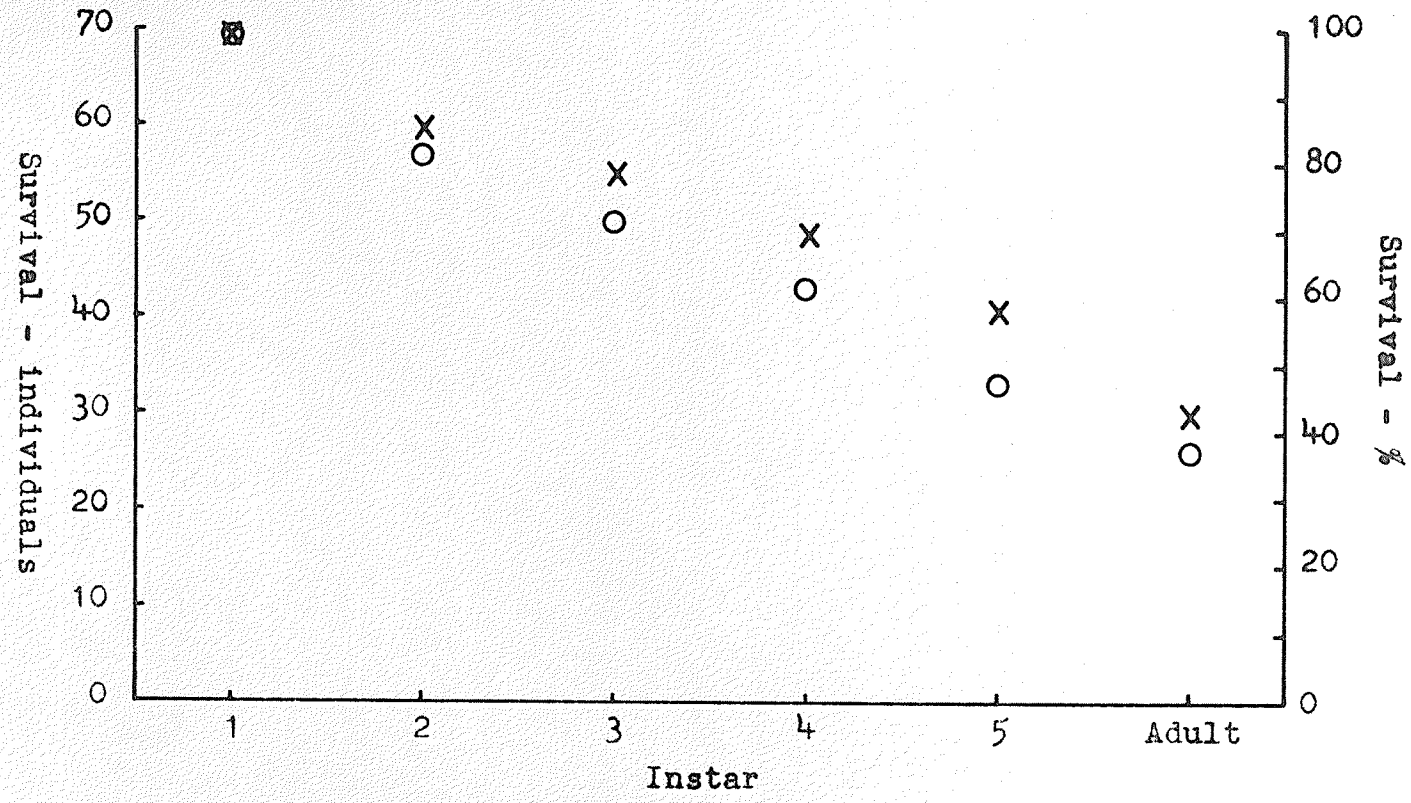

Fig. 9.- Survival, by instars, of nymphs fed diet containing 0.72% hyoscyamine hydrochloride. This treatment did not significantly affect the number becoming adult.

X Treatment O Control

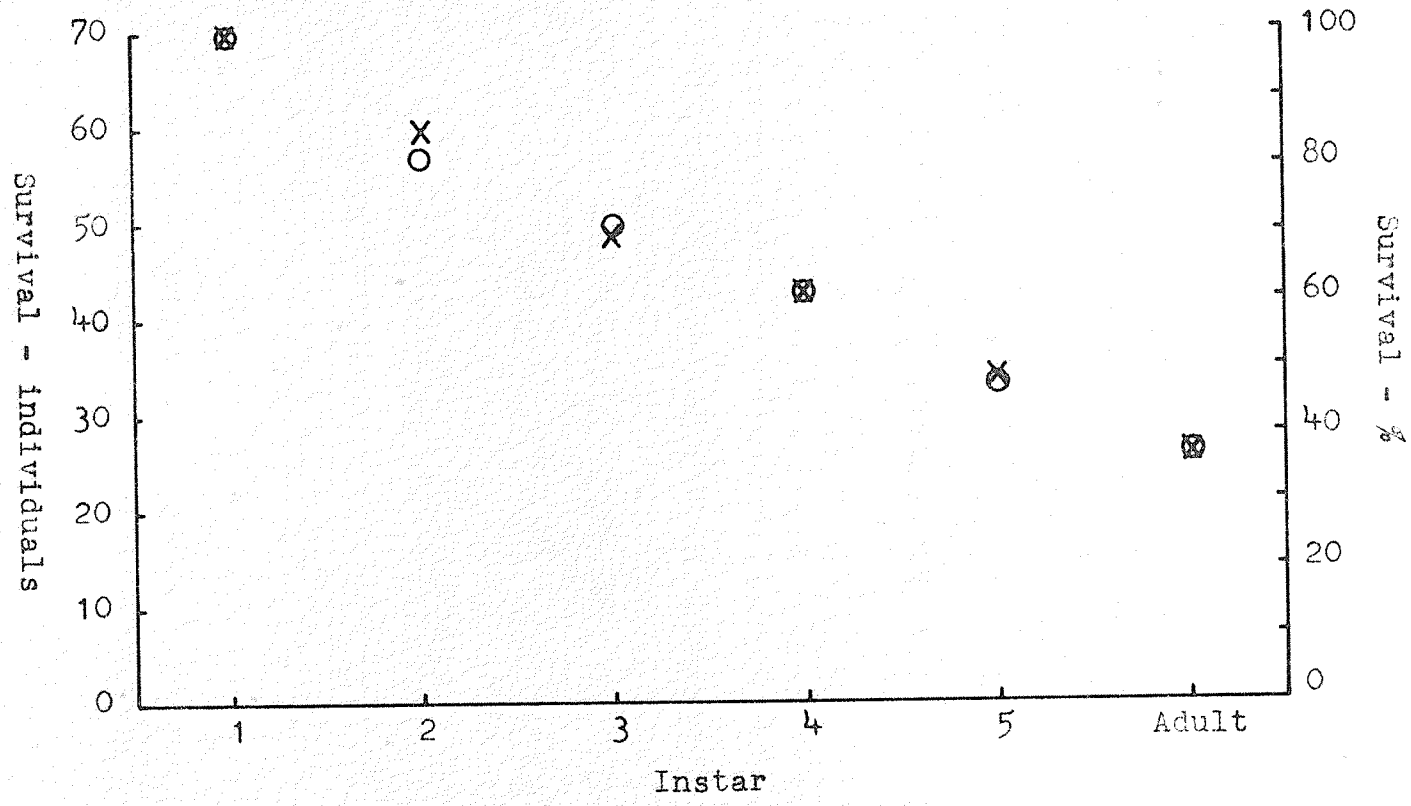

Fig. 10 .- Survival, by instars, of nymphs fed diet containing 0.59% arbutin. This treatment did not significantly affect the number becoming adult.

X Treatment O Control

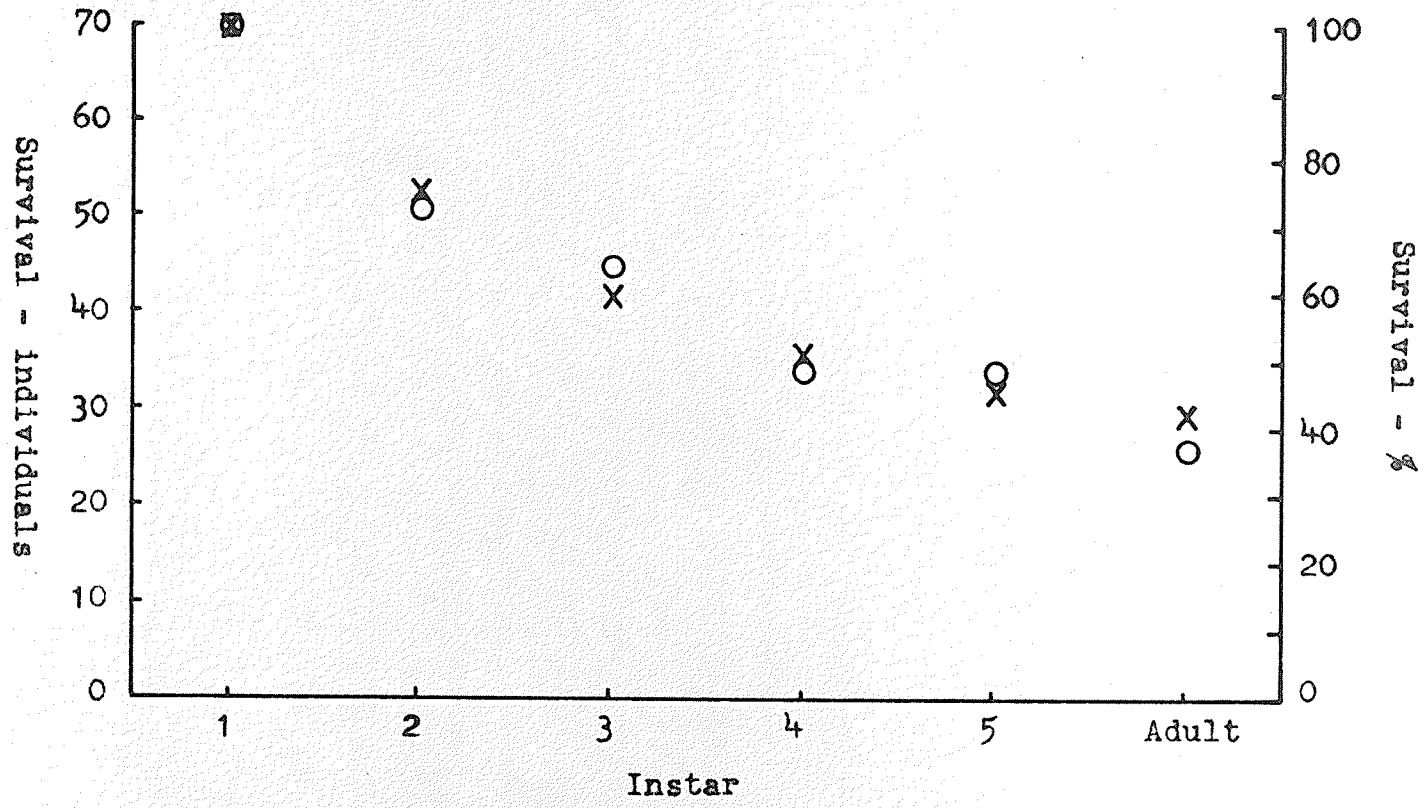

Fig. 11.- Survival, by instars, of nymphs fed diet containing 1.67% lobeline sulphate. This treatment did not significantly affect the number becoming adult.

X Treatment O Control

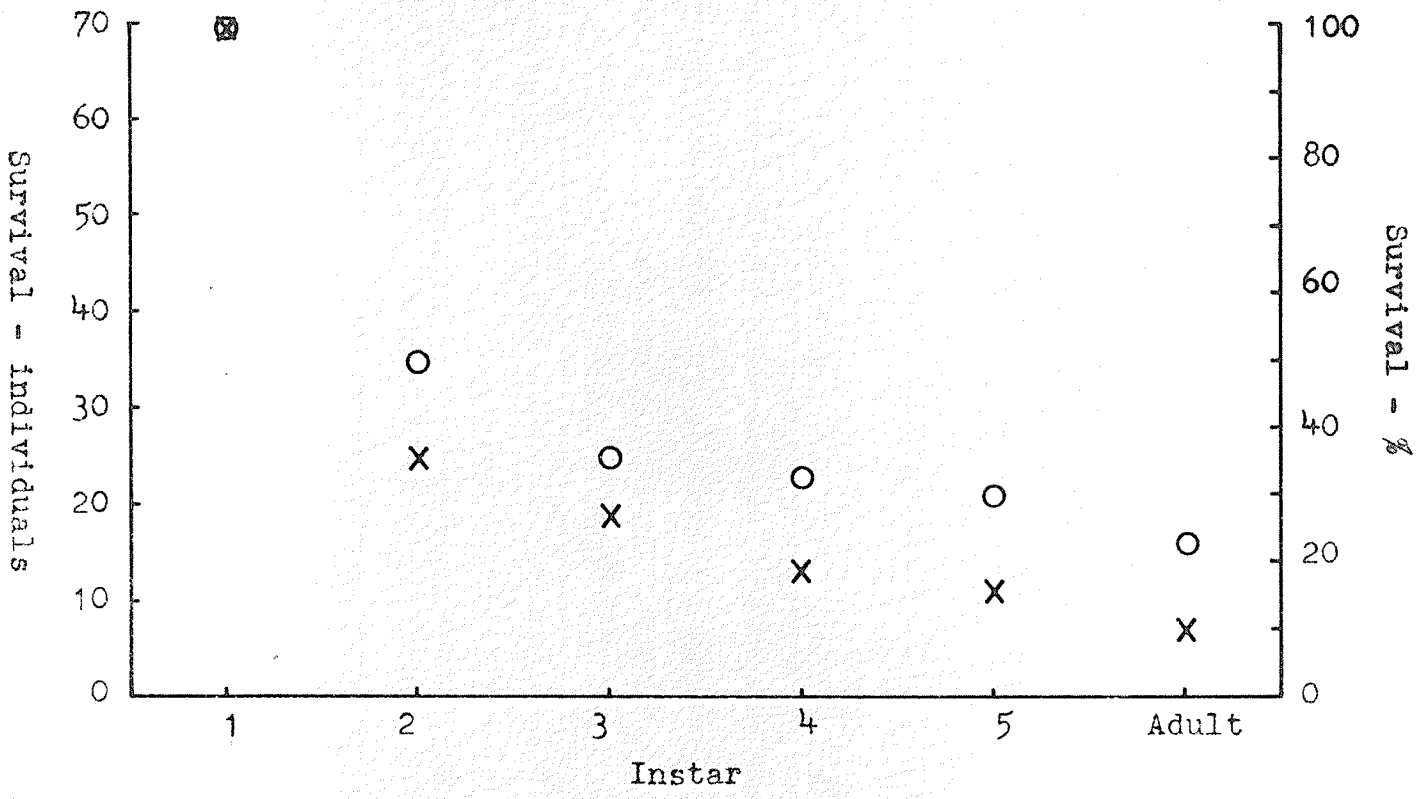

Fig. 12.- Survival, by instars, of nymphs fed diet containing 0.65% indican. This treatment significantly decreased the number becoming adult.

X Treatment O Control

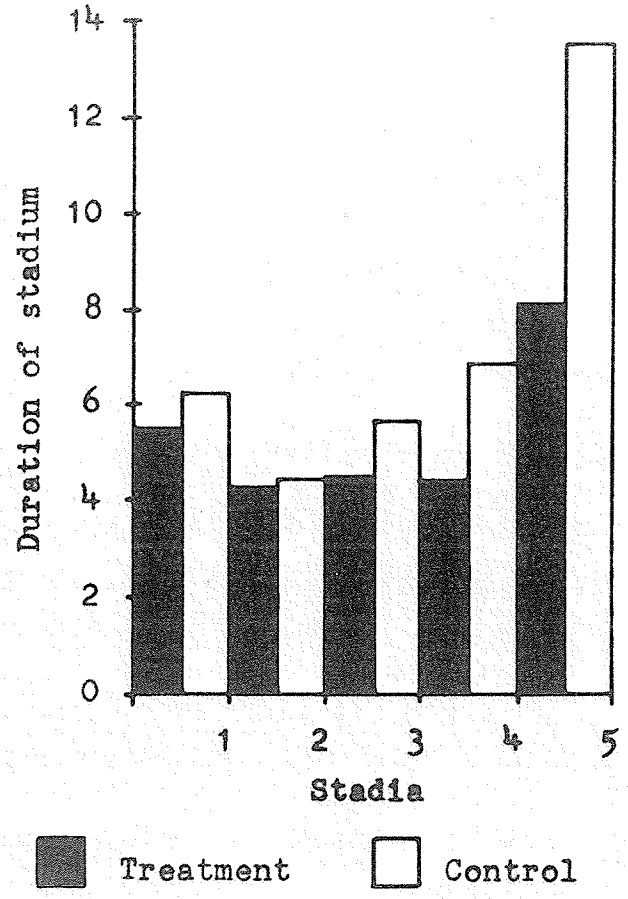

Fig. 13.- Mean durations of stadia for nymphs fed diet containing tigogenin and for control insects. Tigogenin increased survival and rate of weight gain.

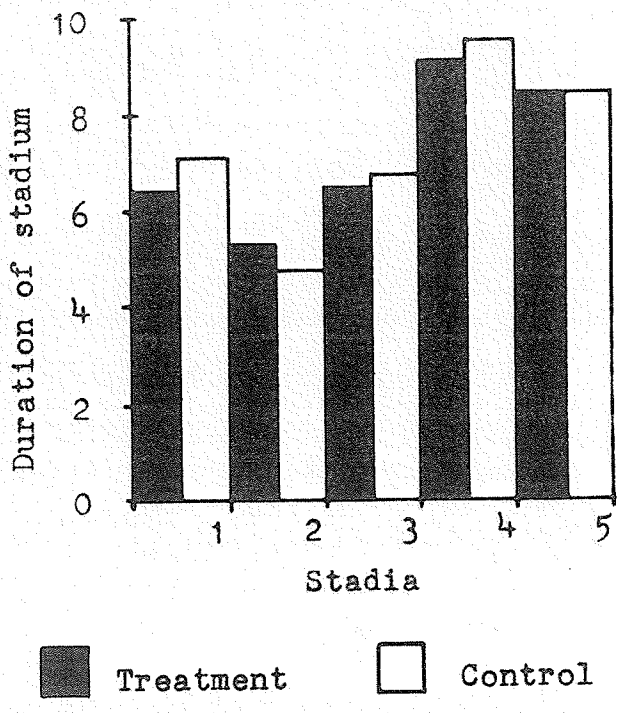

Fig. 14.- Mean durations of stadia for nymphs fed diet containing hordenine sulphate and for control insects. Hordenine sulphate had no effect on survival or rate of weight gain.

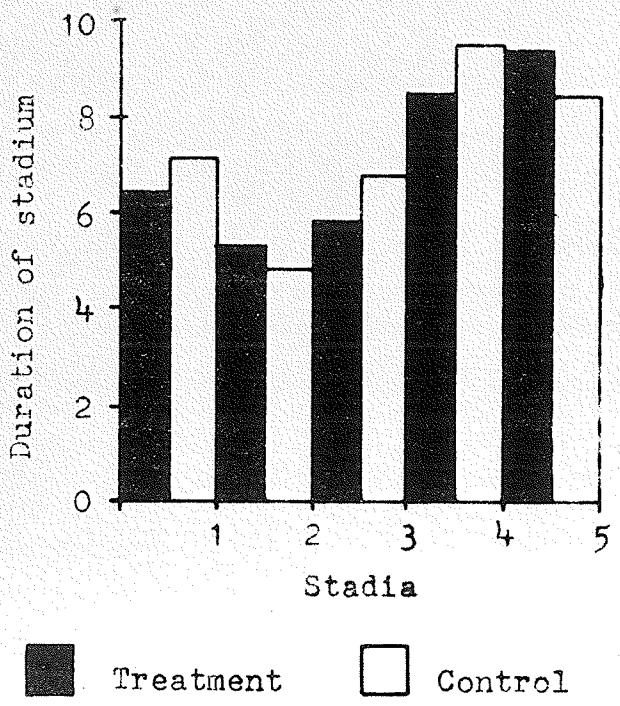

Fig. 15.- Mean durations of stadia for nymphs fed diet containing hyoscyamine hydrochloride and for control insects. Hyoscyamine hydrochloride had no effect on survival or rate of weight gain.

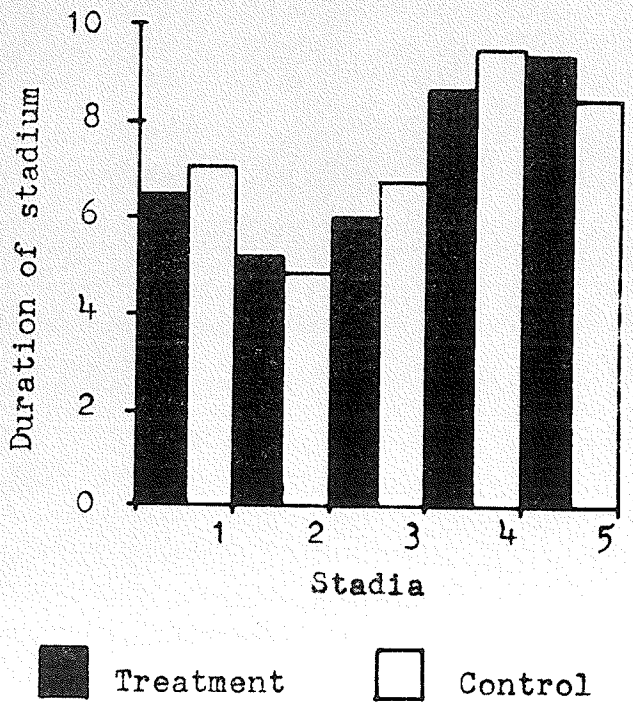

Fig. 16.- Mean durations of stadia for nymphs fed diet containing arbutin and for control insects. Arbutin had no effect on survival or rate of weight gain.

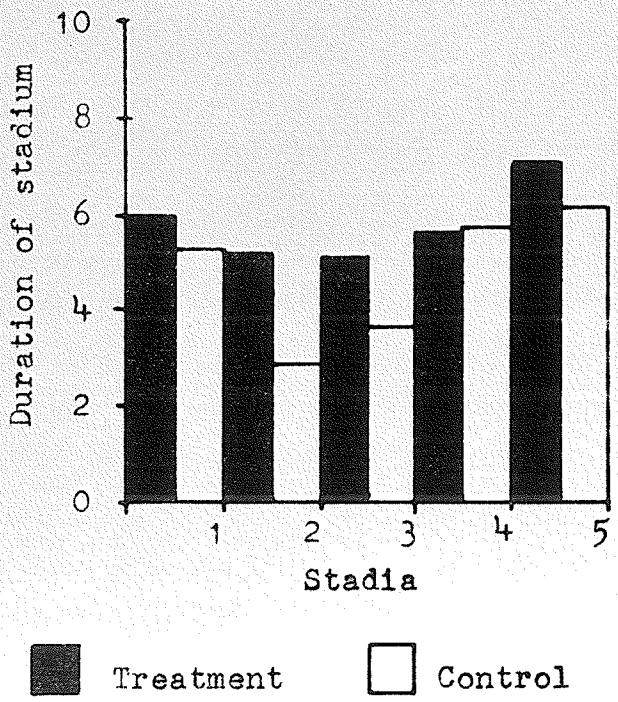

Fig. 17.- Mean durations of stadia for nymphs fed diet containing lobeline sulphate and for control insects. Lobeline sulphate had no effect on survival but decreased rate of weight gain.

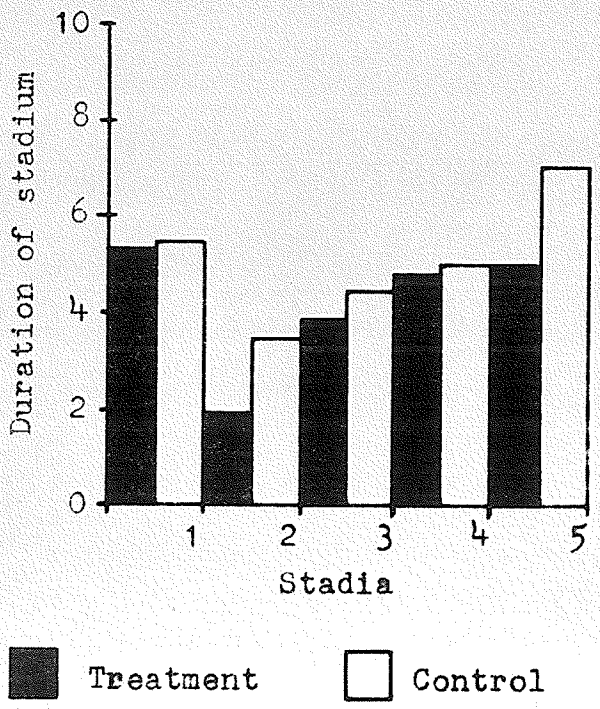

Fig. 18.- Mean durations of stadia for nymphs fed diet containing indican and for control insects. Indican decreased survival and rate of weight gain.

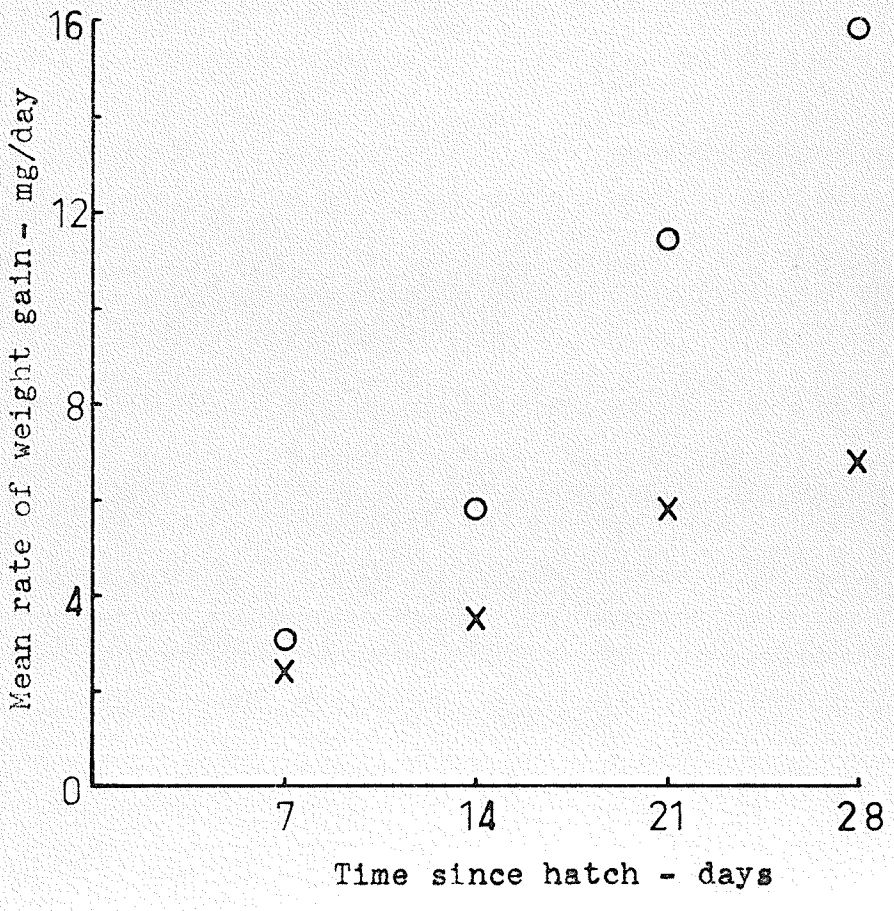

Fig. 19 .- Mean rate of weight gain for nymphs fed diet containing 1.0% tigogenin. This treatment significantly increased the rate of weight gain.

O Treatment X Control

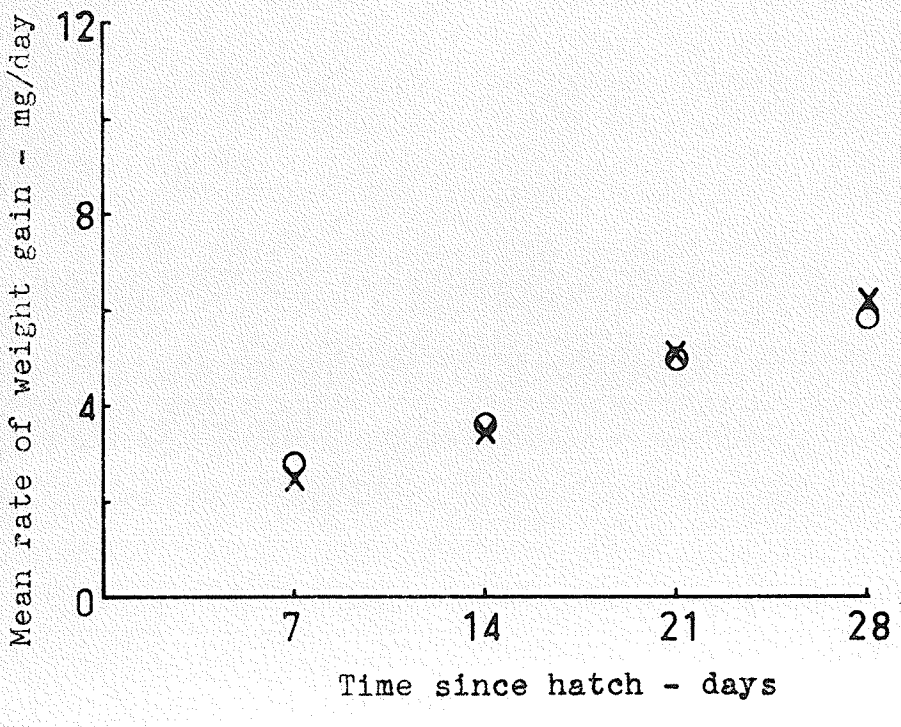

Fig. 20 .- Mean rate of weight gain for nymphs fed diet containing 1.0% hordenine sulphate. This treatment did not significantly affect the rate of weight gain.

○ Treatment × Control

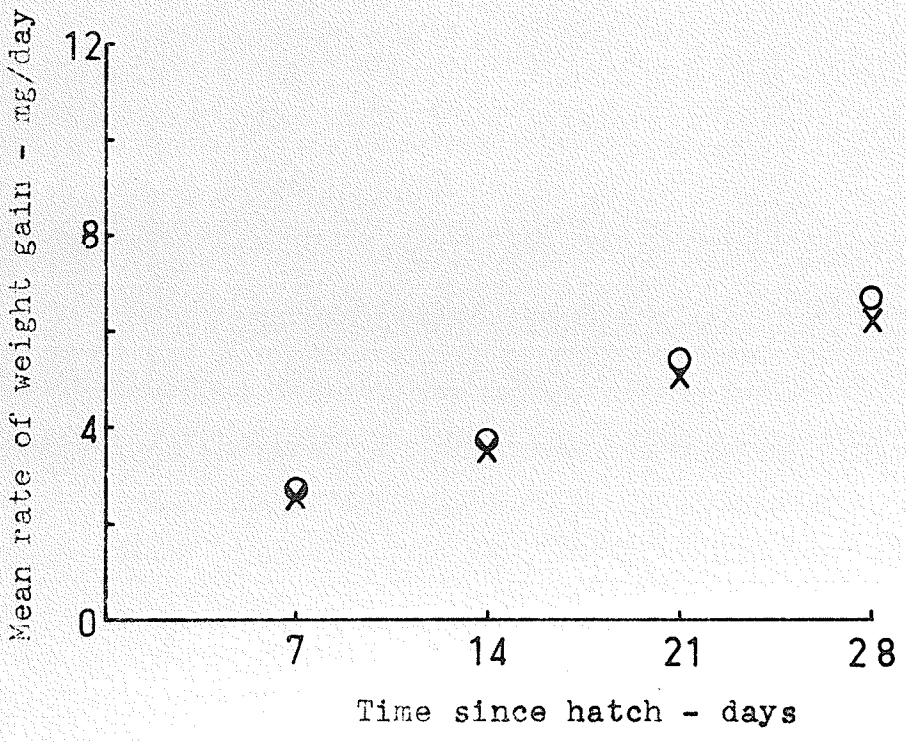

Fig. 21.- Mean rate of weight gain for nymphs fed diet containing0.72% hyoscyamine hydrochloride. This treatment did not significantly affect the rate of weight gain.

O Treatment X Control

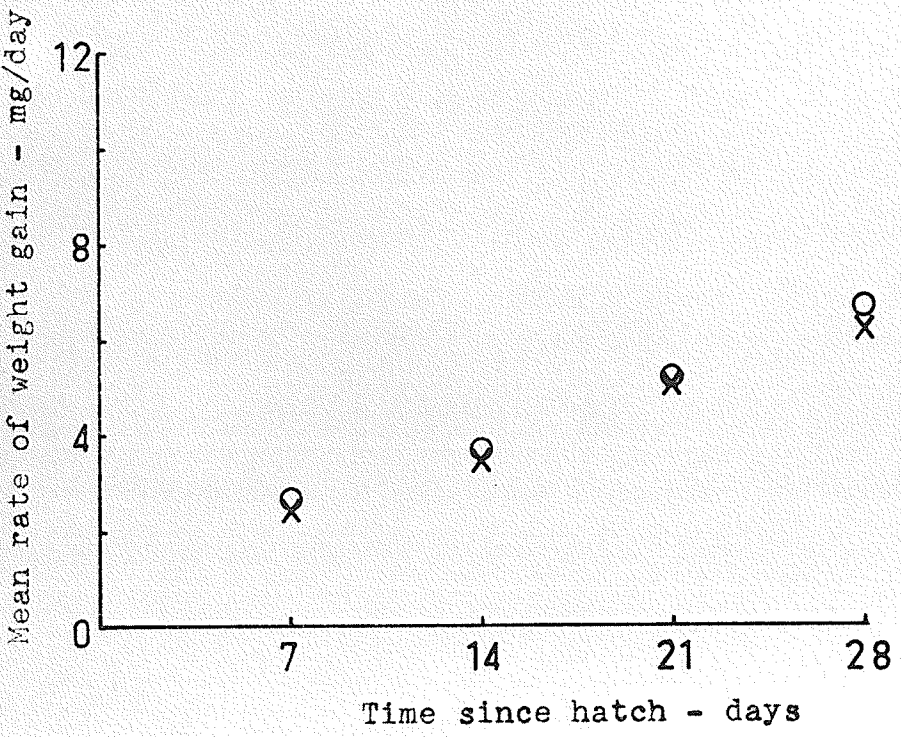

Fig. 22.- Mean rate of weight gain for nymphs fed diet containing 0.59% arbutin. This treatment did not significantly affect the rate of weight gain.

O Treatment X Control.

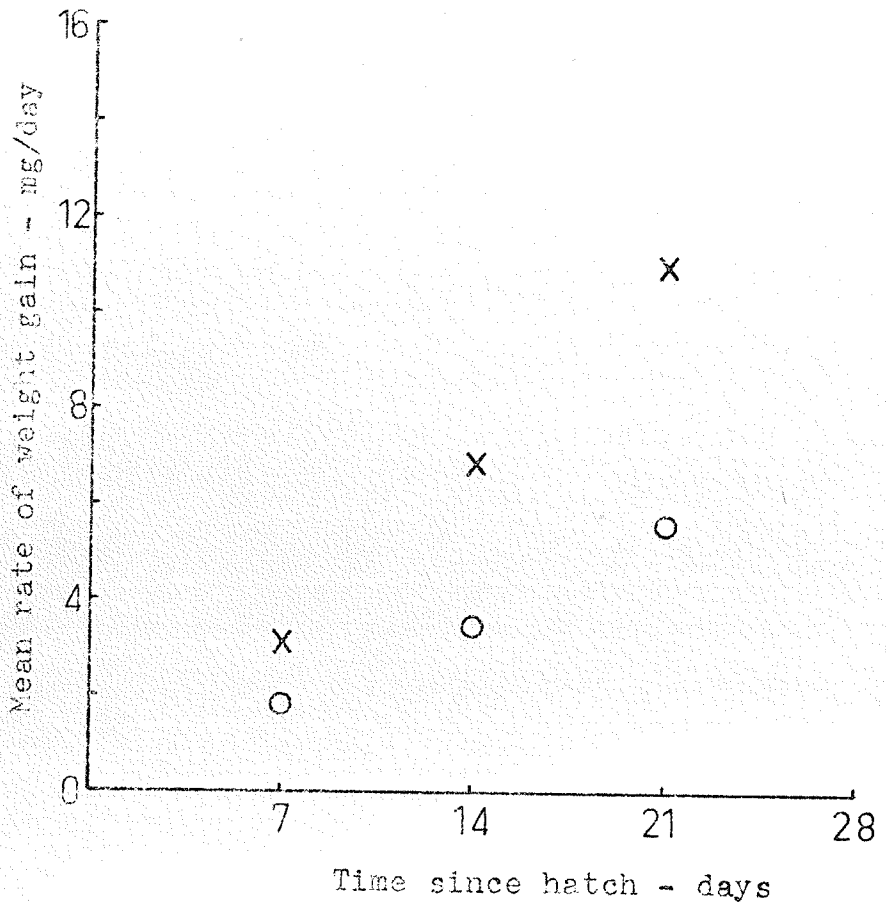

Fig.23 .- Mean rate of weight gain for nymphs fed diet containing 1.67% lobeline sulphate. This treatment singificantly decreased the rate of weight gain.

O Treatment X Control

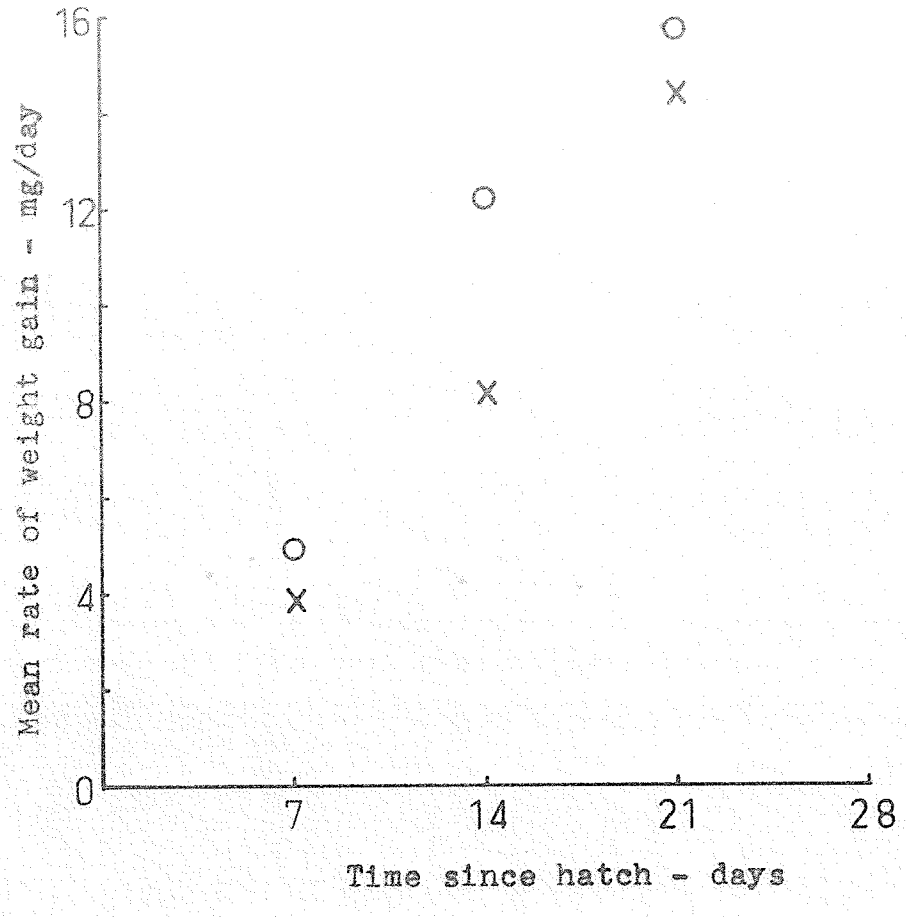

Fig. 24.- Mean rate of weight gain for nymphs fed diet containing 0.65% indican. This treatment did not significantly affect the rate of weight gain.

O Treatment X Control

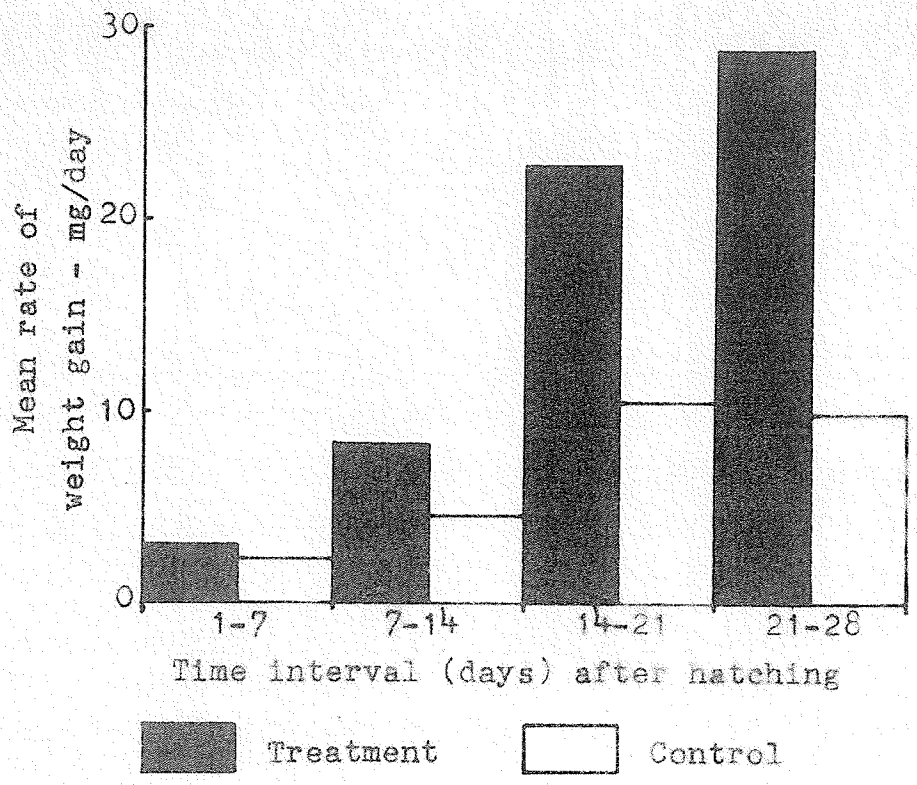

Fig. 25.- Mean rate of weight gain over successive seven-day intervals for nymphs fed diet containing 1.0% tigogenin.

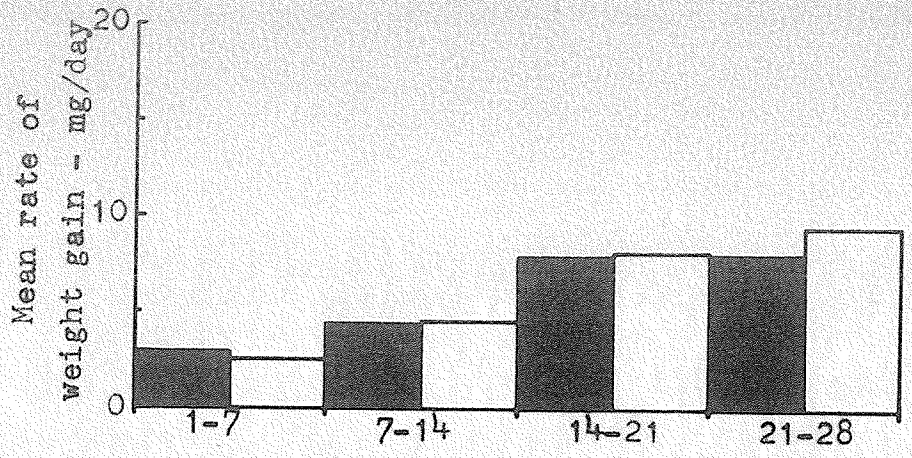

Fig. 26 .- Hordenine sulphate (1.0%)

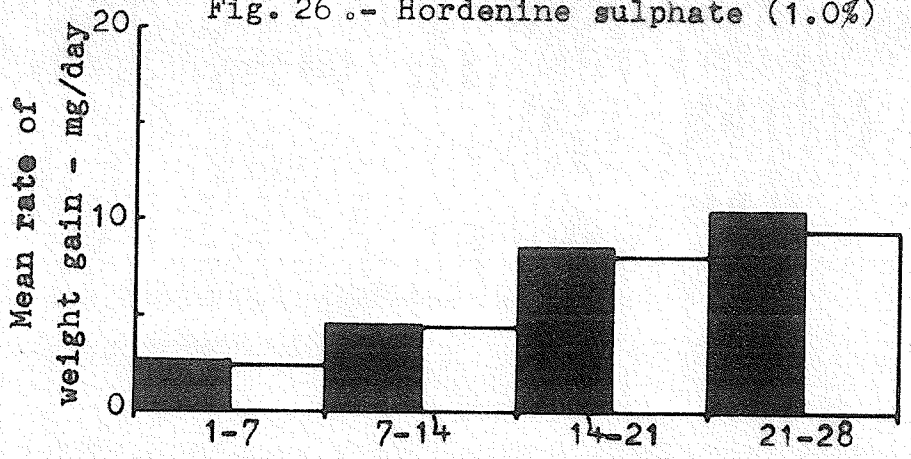

Fig. 27 .- Hyoscyamine hydrochloride (0.72%)

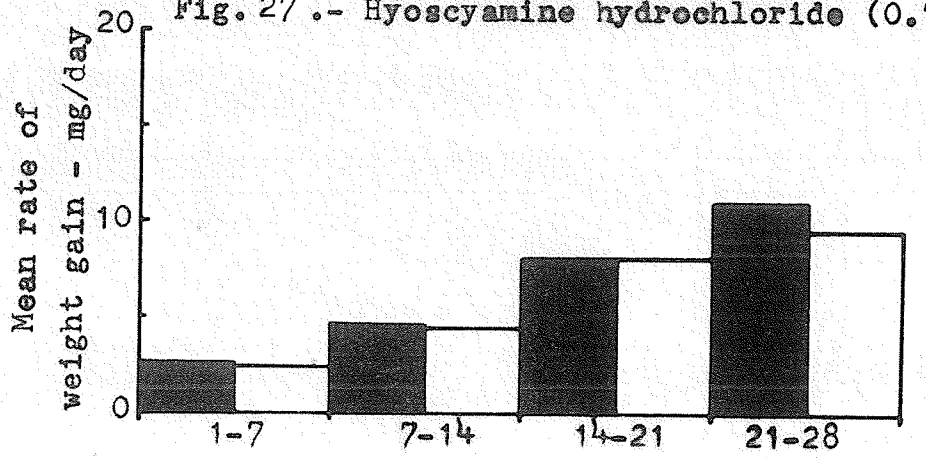

Fig. 28 .- Arbutin (0.59%)

Treatment Control

Figs. 26 to 28.- Mean rate of weight gain over successive seven-day intervals for nymphs fed diet containing hordenine sulphate, hyoscyamine hydrochloride, or arbutin.

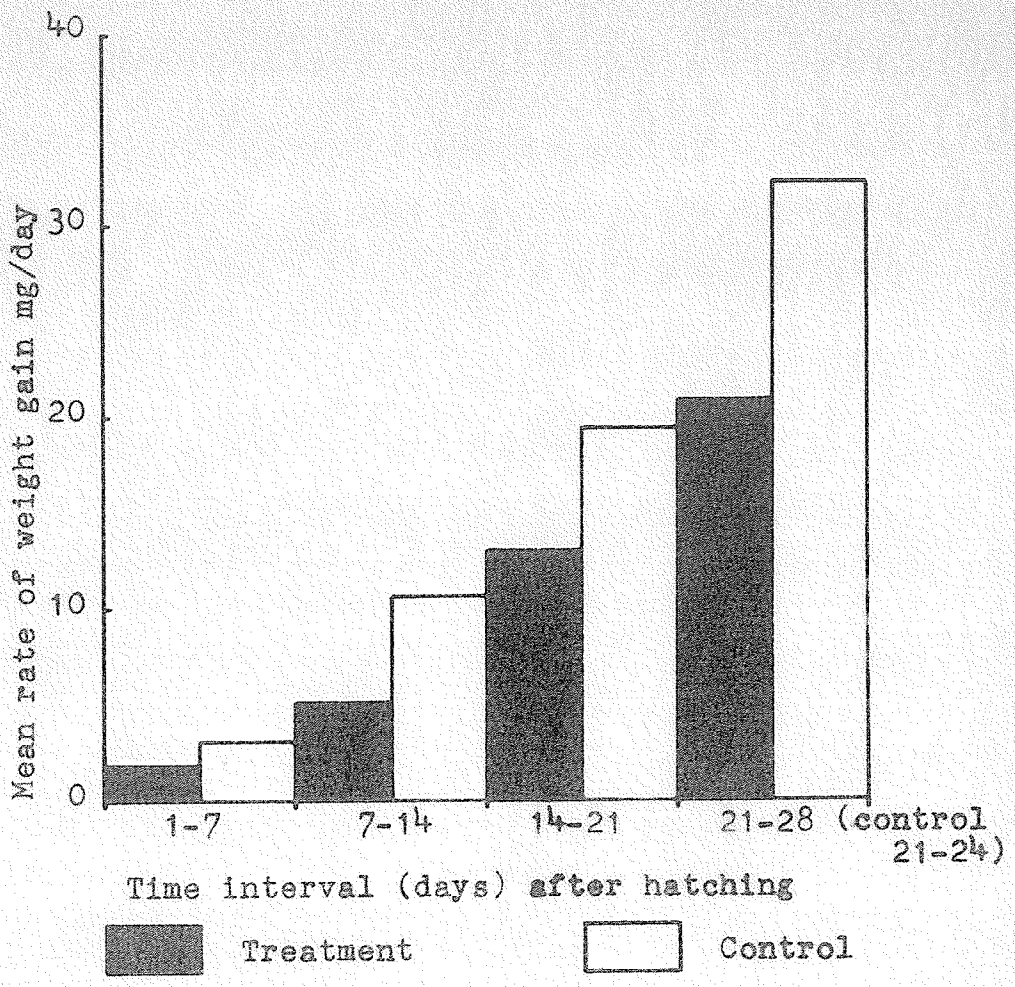

Fig.29 .- Mean rate of weight gain over successive seven day intervals for nymphs fed diet containing 1.67% lobeline sulphate.

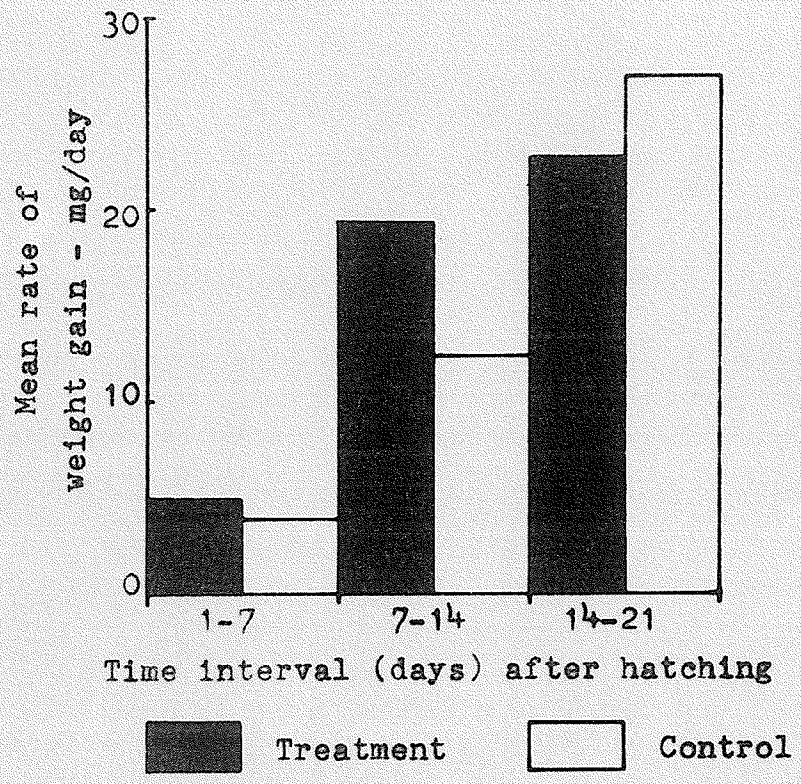

Fig.30 .- Mean rate of weight gain over successive seven day intervals for nymphs fed diet containing 0.65% indican.

II. INFLUENCE OF TEST CHEMICALS ON FEEDING BEHAVIOUR

(a) Inhibition of drinking response.

(i) Results. Data on the inhibition of drinking response are presented in Table XV. Most of these results are for tests performed with solutions of one-tenth M concentration or emulsions of equivalent concentration. Concentration was varied in a small number of tests but had little effect on results obtained and this factor was not further investigated.

Figure 31 compares effects of test chemicals on drinking response with their effects on survival recorded in growth experiments.

(ii) Discussion. In discussing these data it has been postulated that most chemicals which reduced survival, or were toxic in growth experiments would, on teleological grounds, be repellent to the insect, whereas most of those which stimulated or did not affect survival would not be repellent.

Figure 31 indicates that results of experiments with five chemicals, (gramine, arbutin, β-sitosterol, indican, and digitonin), were in accord with this hypothesis, results with four chemicals (santonine, lupinine, hydrastine hydrochloride, and veratrine), were inconclusive either in growth experiments or in these tests, and that results for

four chemicals, (lobeline sulphate, hordenine sulphate, hyoscamine hydrochloride, and saponin), were inconsistent with the hypothesis. Of the latter group, saponin is the only chemical which is not a salt. Solutions of inorganic salts have been reported to stimulate special sensory neurons in diptera (Hodgson 1958). If neurons which are particularly sensitive to salts occur on the distal segments of the palps of M. bivittatus then unexpected repellent effects observed in these tests may have been due to the chemicals being tested as salts and not to any property inherent in the parent alkaloid.

The feeding behaviour of M. bivittatus was investigated more thoroughly in feeding preference experiments. These experiments are discussed in the next section.

(b) Feeding preference experiments.

(i) Results. Photographs of portions of wafers uneaten after three days exposure to fifth-instar nymphs of M. bivittatus are shown in Figures 32 to 43. Feeding was evident on all wafers except those containing lobeline sulphate or nornicotine dipicrate (Figs. 37, 40). Both these chemicals belong to the pyridine group of alkaloids (Henry 1949).

Analysis of data on the volumes of uneaten portions of wafers indicated that either equal feeding occurred on

wafers containing a test chemical and on wafers of control diet, or feeding was significantly less on wafers containing a test chemical. Addition of a test chemical never resulted in significantly more feeding (Table XVI).

Figure 44 compares data from feeding preference experiments with data on survival and rate of weight gain obtained in growth experiments.

(ii) Discussion. Table XVI indicates that M. bivittatus exercises marked discrimination in selection of food. Approximately half the test chemicals significantly reduced feeding while the remainder had no effect on feeding behaviour. These effects suggest that this insect possesses sensory receptors capable of perceiving at least those chemicals which reduced feeding.

Feeding, which ranged from slight nibbling to marked feeding, could be detected on practically all diets containing a test chemical (Figs. 32 to 43). This indicates that detection of a chemical which reduces feeding is associated with biting. Hence sensory receptors may be located on the mouthparts or within the buccal cavity. Results of tests on inhibition of drinking response suggest that some sensory receptors are located on the distal segments of the maxillary and labial palps.

From a behavioural aspect plant chemicals may constitute feeding stimulants, (which increase feeding by a phytophagous insect), they may have no effect on feeding behaviour, or they may be feeding inhibitors, (which decrease feeding by phytophagous insects).

In feeding preference experiments none of the twenty test chemicals resulted in greater feeding on treated than on control wafers. However approximately half the chemicals resulted in no significant difference in feeding on treated and on control wafers, and approximately half resulted in lesser feeding on treated than on control wafers (Table XVI). Control wafers contained two known feeding stimulants for M. bivittatus, sucrose (Thorsteinson 1960a) and lecithin (Thorsteinson and Nayar 1963), and hence, addition of approximately half the test chemicals to wafers of a diet containing feeding stimulants resulted in it being non-preferred when compared with wafers of control diet. These chemicals, gramine, hordenine sulphate, hyoscyamine hydrochloride, lobeline sulphate, veratrine, lupinine, diosigenin, digitonin, and nornicotine dipicrate, should be regarded as feeding inhibitors for M. bivittatus.

Interpretations of the effects of test chemicals on the feeding behaviour of M. bivittatus from data on feeding preference experiments and from data on drinking response tests are in substantial agreement. However, gramine, lupinine, and indican inhibited drinking by adults but did not prevent feeding by fifth-instar nymphs.

TABLE XV

INHIBITION OF DRINKING RESPONSE WHEN CHEMICALS WERE APPLIED TO THE PALPS OF *M. BIVITTATUS*

Test chemical	Number of insects		Quartile distribution of inhibition		
	Responding to water	Response to water inhibited by chemical	0-25	26-75	76-100
Gramine#	10	0	+		
Hordenine sulphate	10	8			+
Hydrastine hydrochloride	28	13		+	
Hyoscyamine hydrochloride	18	15			+
Lobeline sulphate	10	8			+
Lupinine	19	2	+		
Santonine	9	1	+		
Veratrine	9	5		+	
Arbutin	30	2	+		
Indican	9	7			+
Digitonin	10	9			+
Saponin#	10	0	+		
β-sitosterol	20	1	+		

Results for all chemicals, except those marked #, are for 1/10M solutions, or emulsions of equivalent concentration.

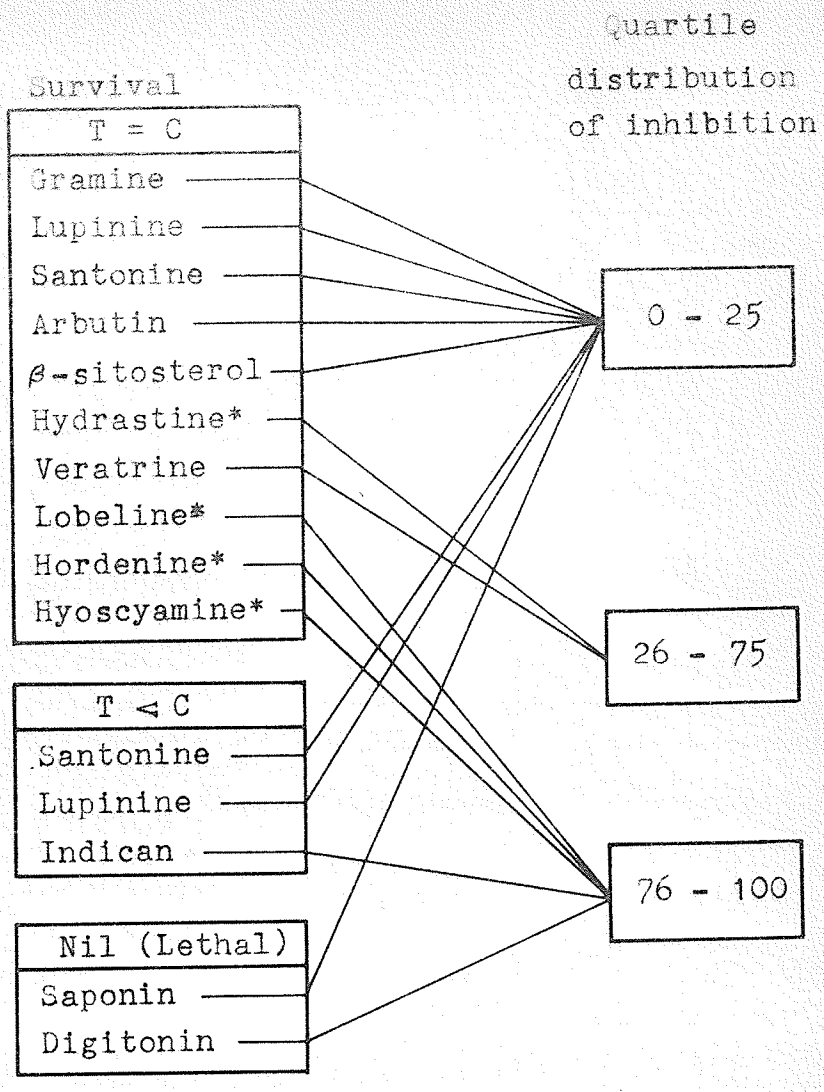

Fig. 31.- Comparison of effects of test chemicals on drinking response and on survival (data from growth experiments).

* Salts of these chemicals were used in all experiments.

T = diet to which test chemical was added.

C = control diet.

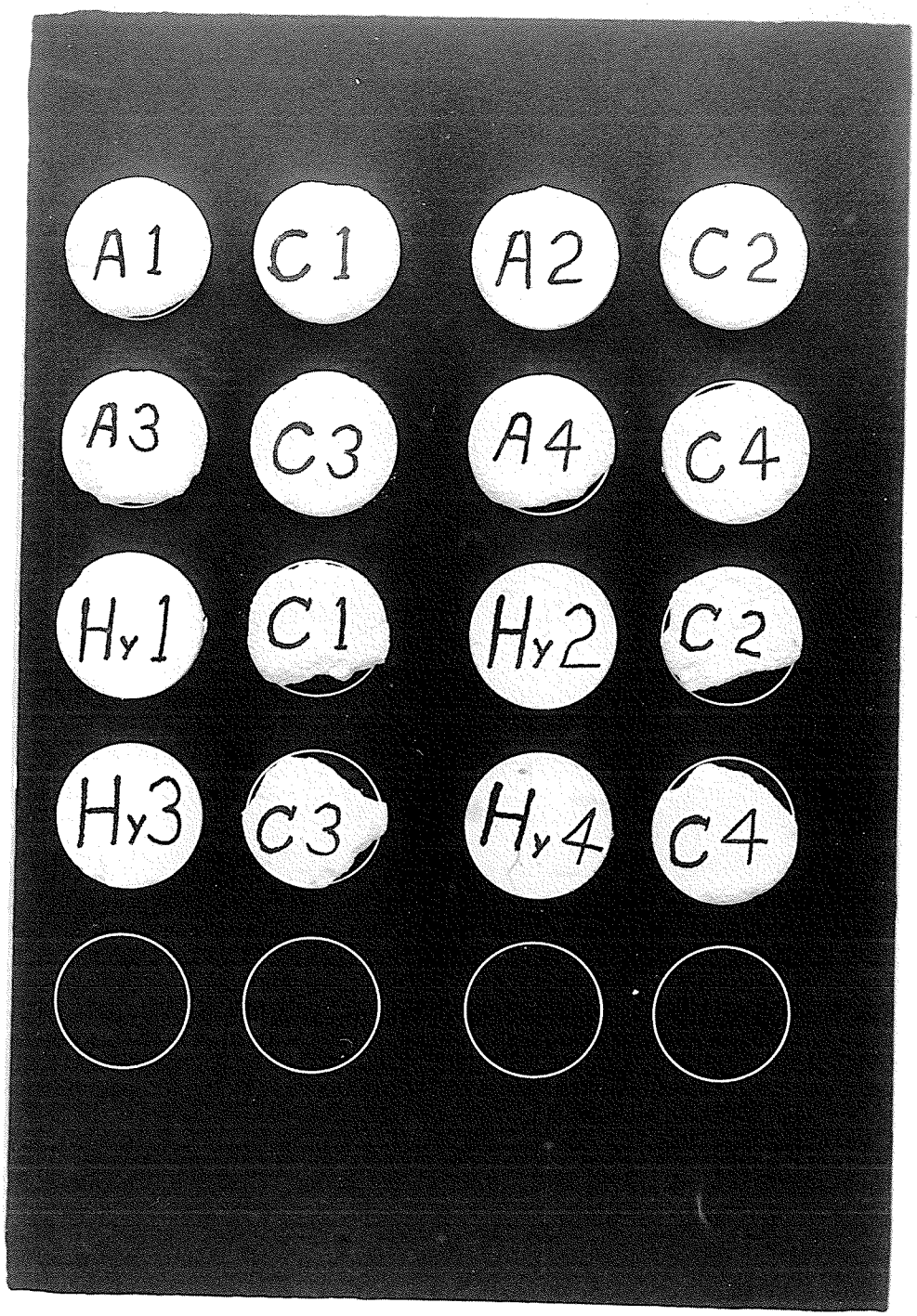

Fig. 32.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing arbutin (A) was not significantly different to feeding on control wafers (C). Feeding on wafers containing hyoscyamine hydrochloride (Hy) was significantly less than on control wafers.

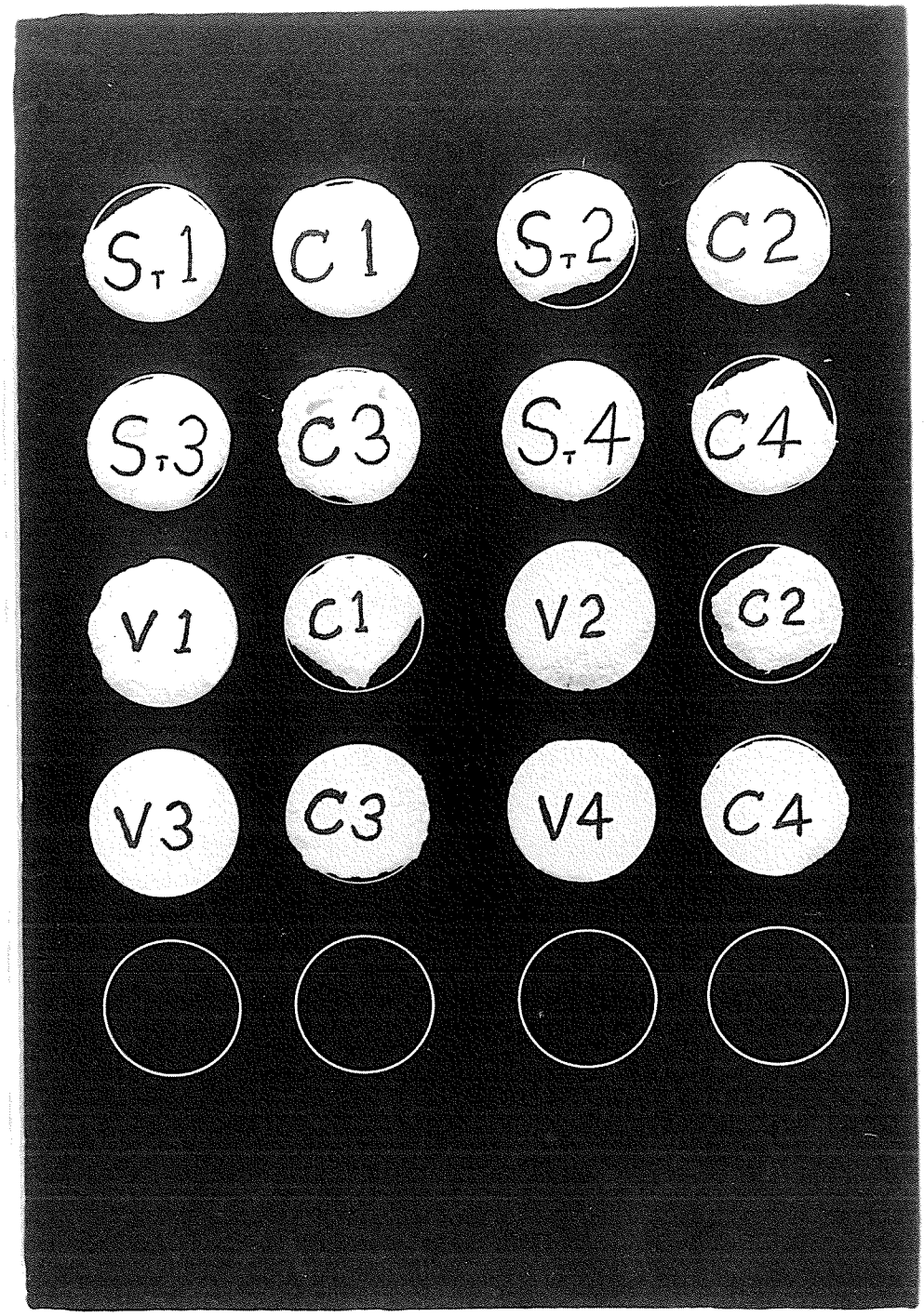

Fig. 33.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing stigmasterol (St) was not significantly different to feeding on control wafers (C). Feeding on wafers containing veratrine (V) was significantly less than on control wafers.

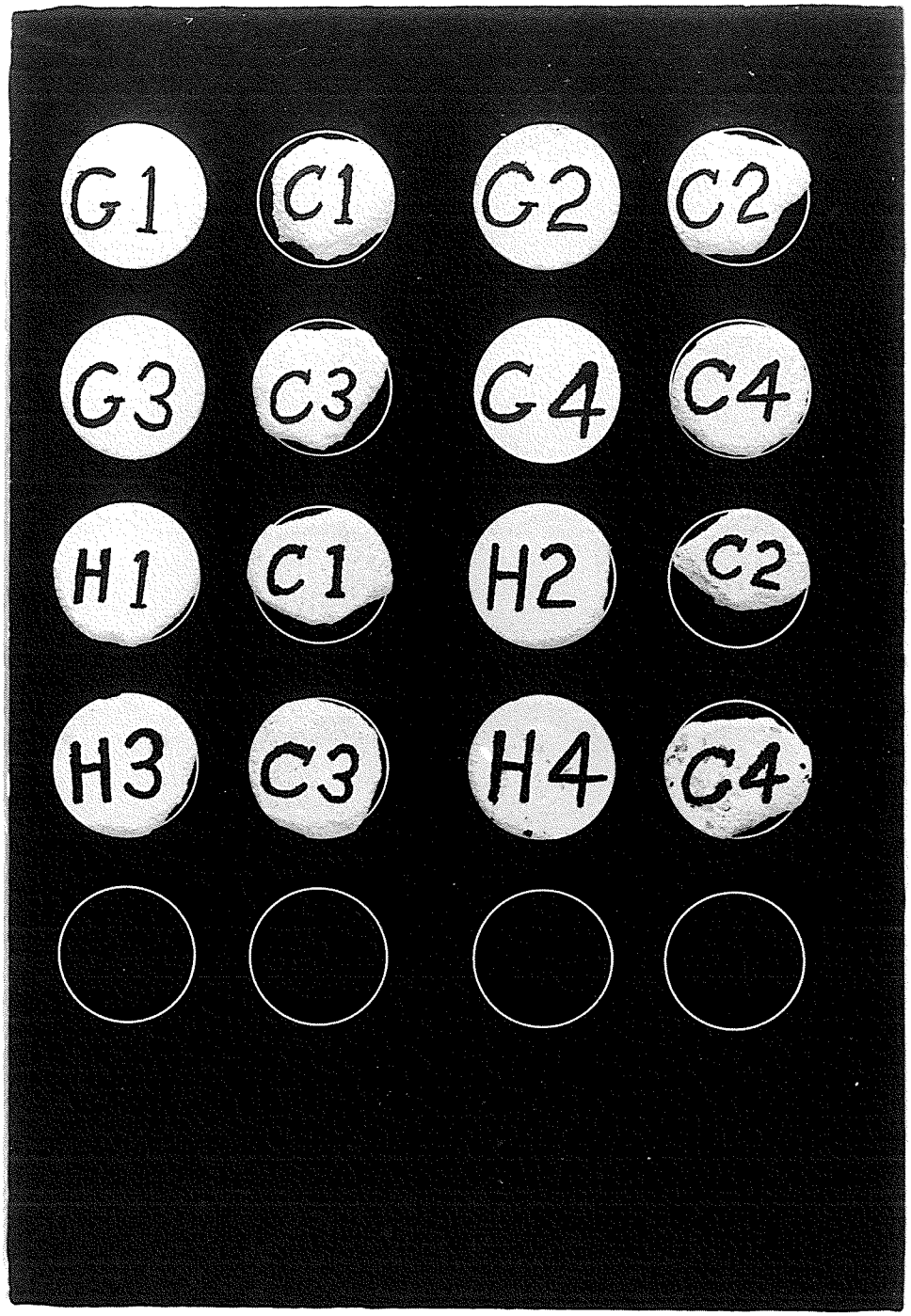

Fig. 34.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing either gramine (G) or hordenine sulphate (H) was significantly less than on control wafers (C).

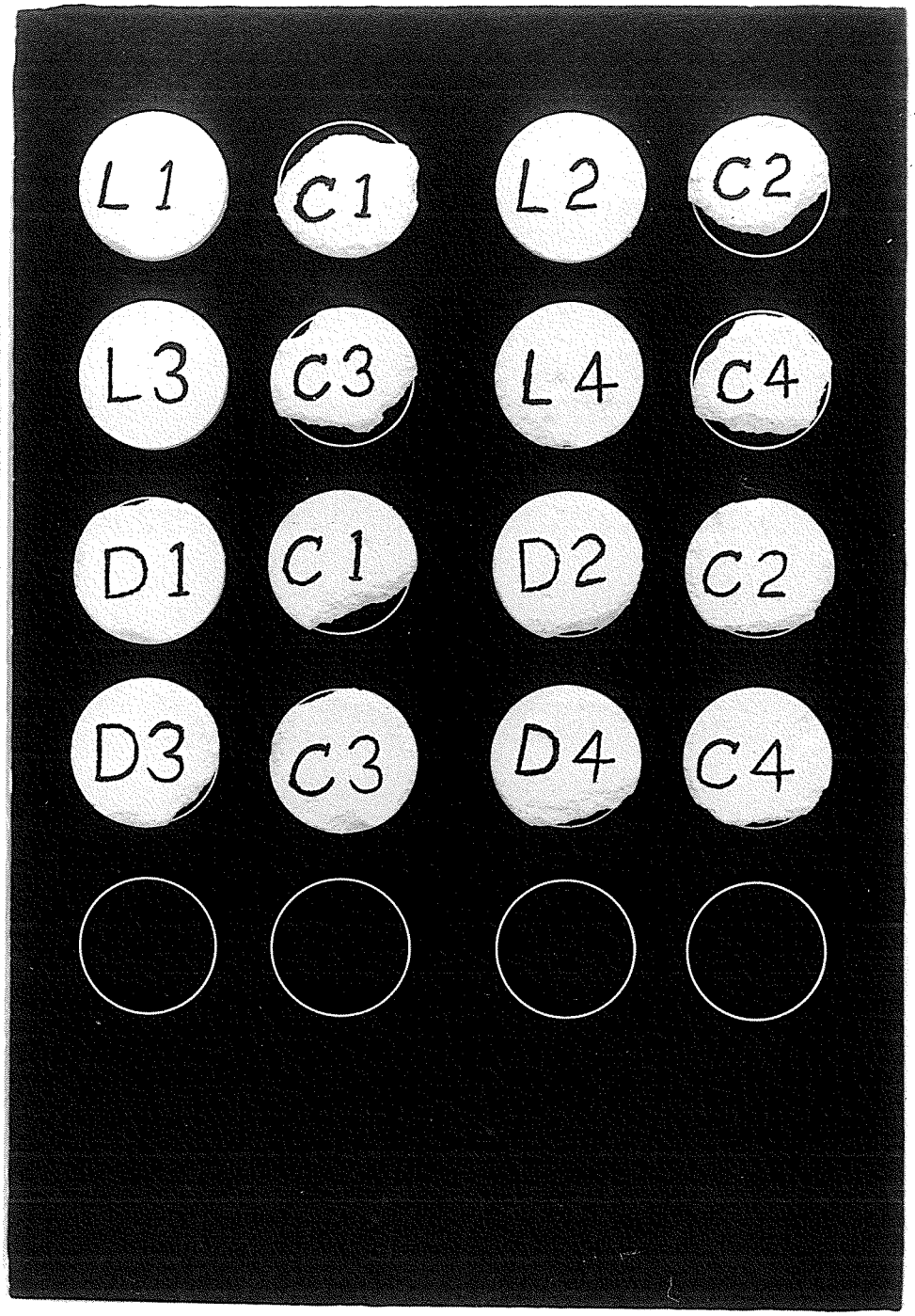

Fig. 35.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing either lupinine (L) or diosgenin (D) was significantly less than feeding on control wafers (C).

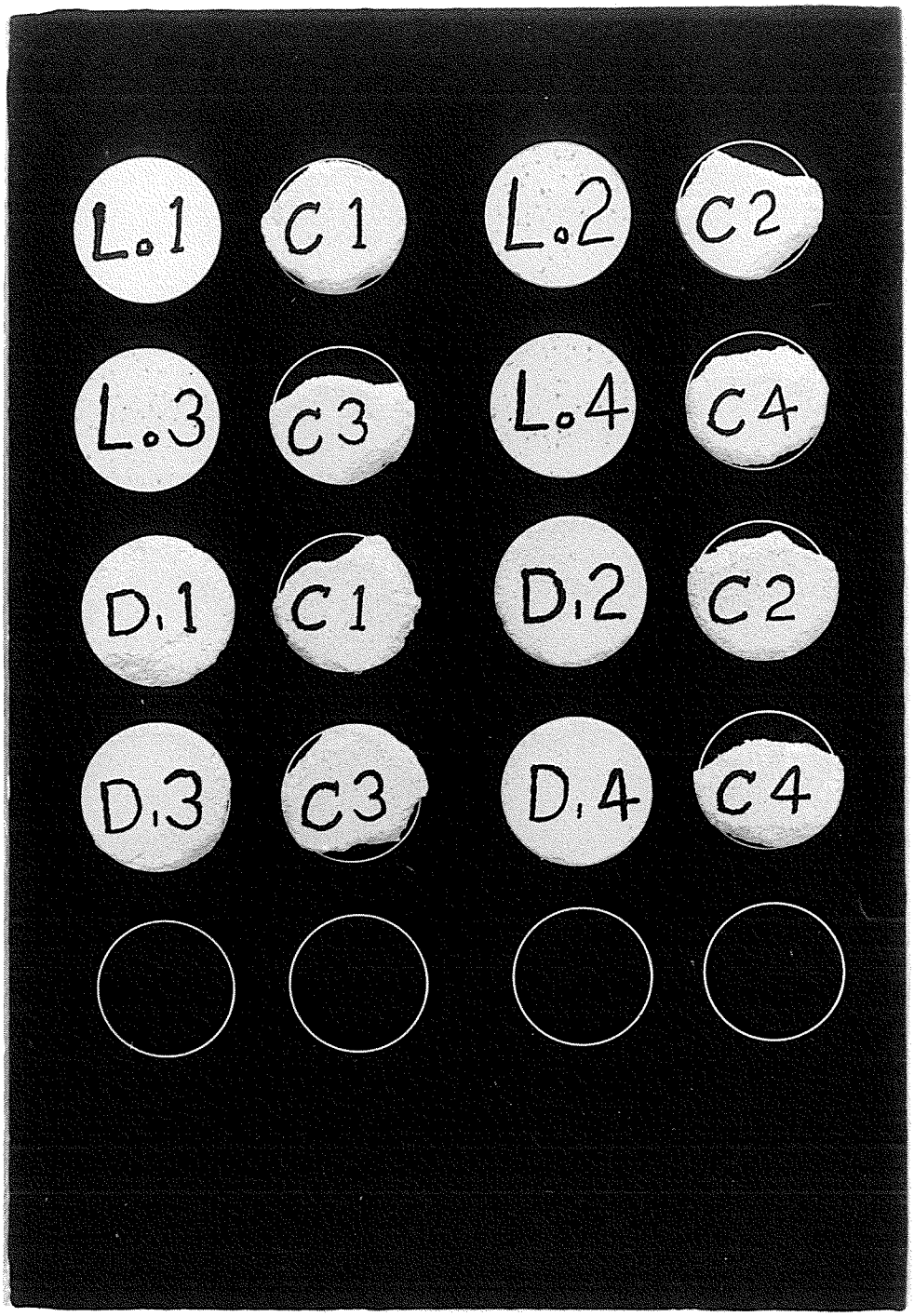

Fig. 36 .- Portions of wafers not eaten in preference experiments. Feeding on wafers containing either lobeline sulphate (Lo) or digitonin (Di) was significantly less than on control wafers (C).

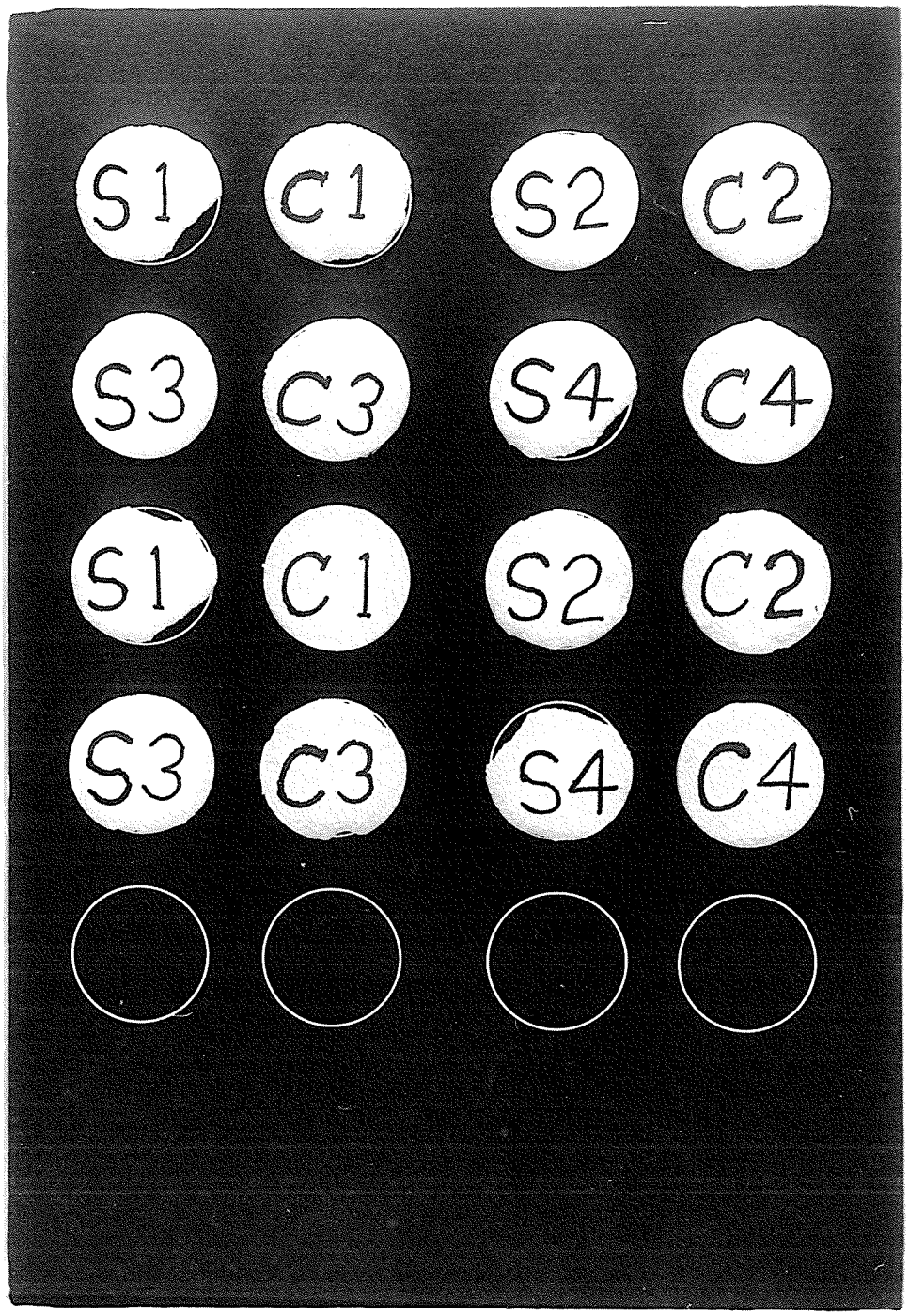

Fig. 37.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing saponin (S) was not significantly different to feeding on control (C).

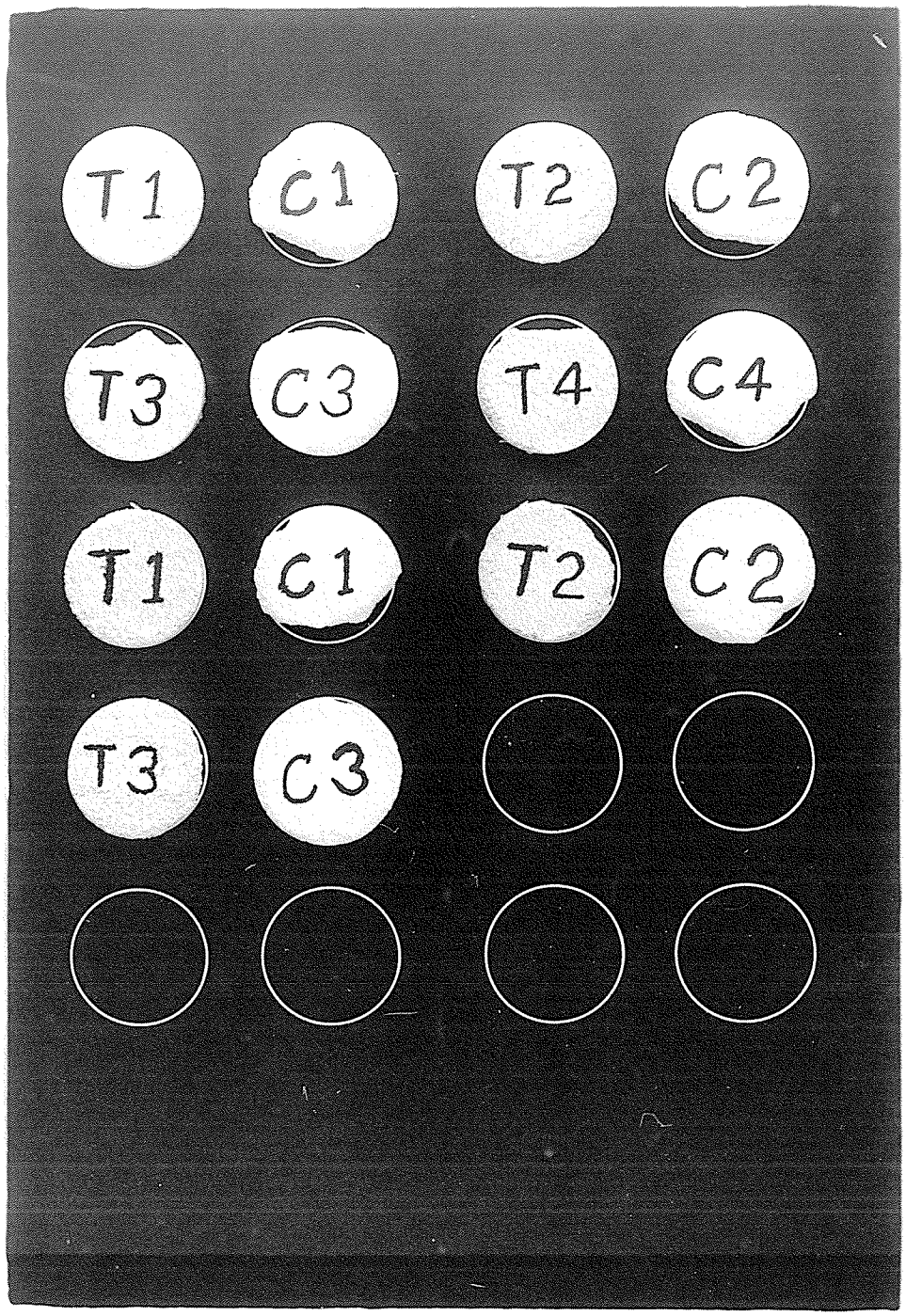

Fig. 38.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing tomatine (T) was not significantly different to feeding on control wafers (C).

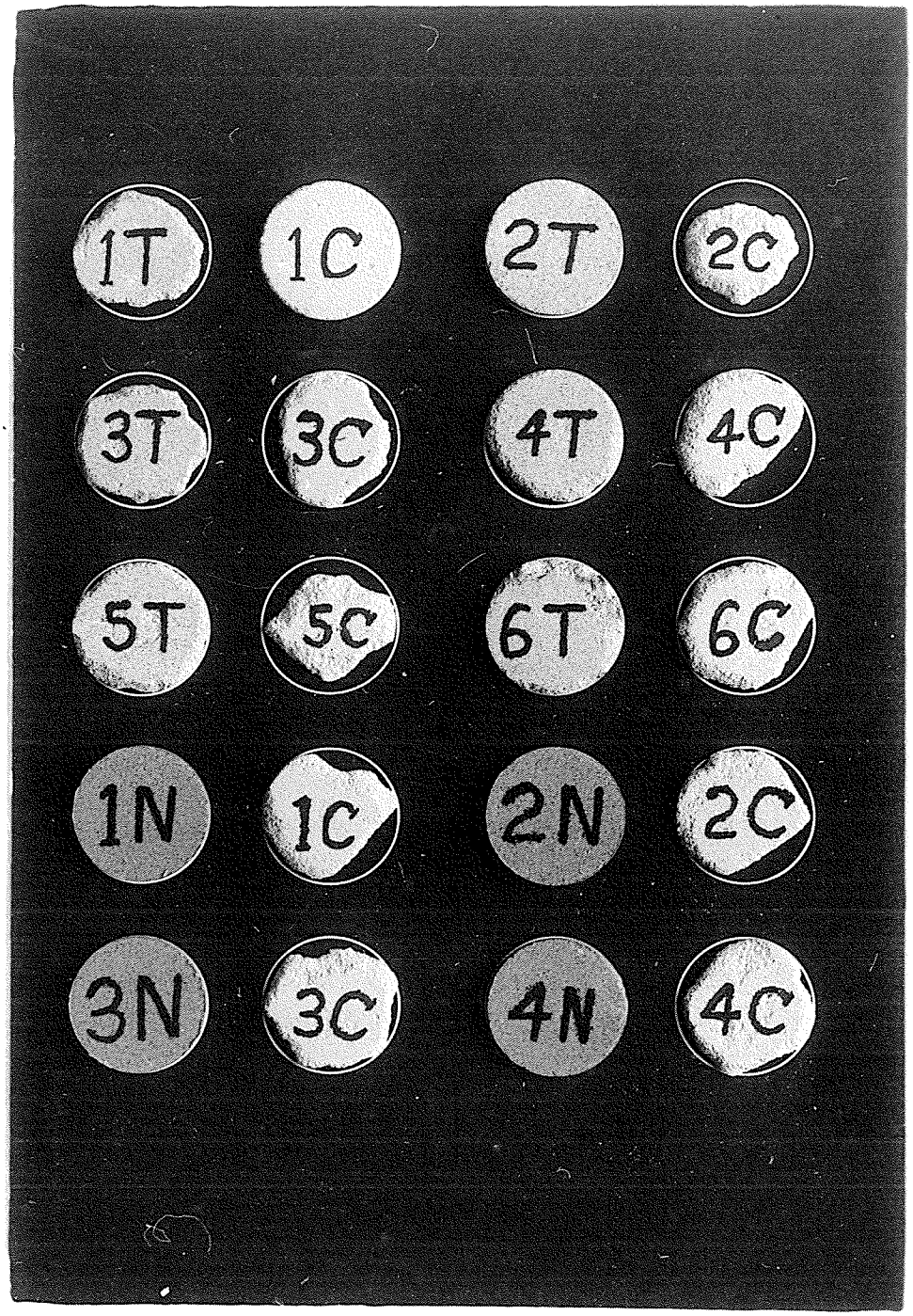

Fig. 39.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing tomatine (T) was not significantly different to feeding on control (C) wafers. Feeding on wafers containing nornicotine dipicrate (N) was significantly less than on control wafers.

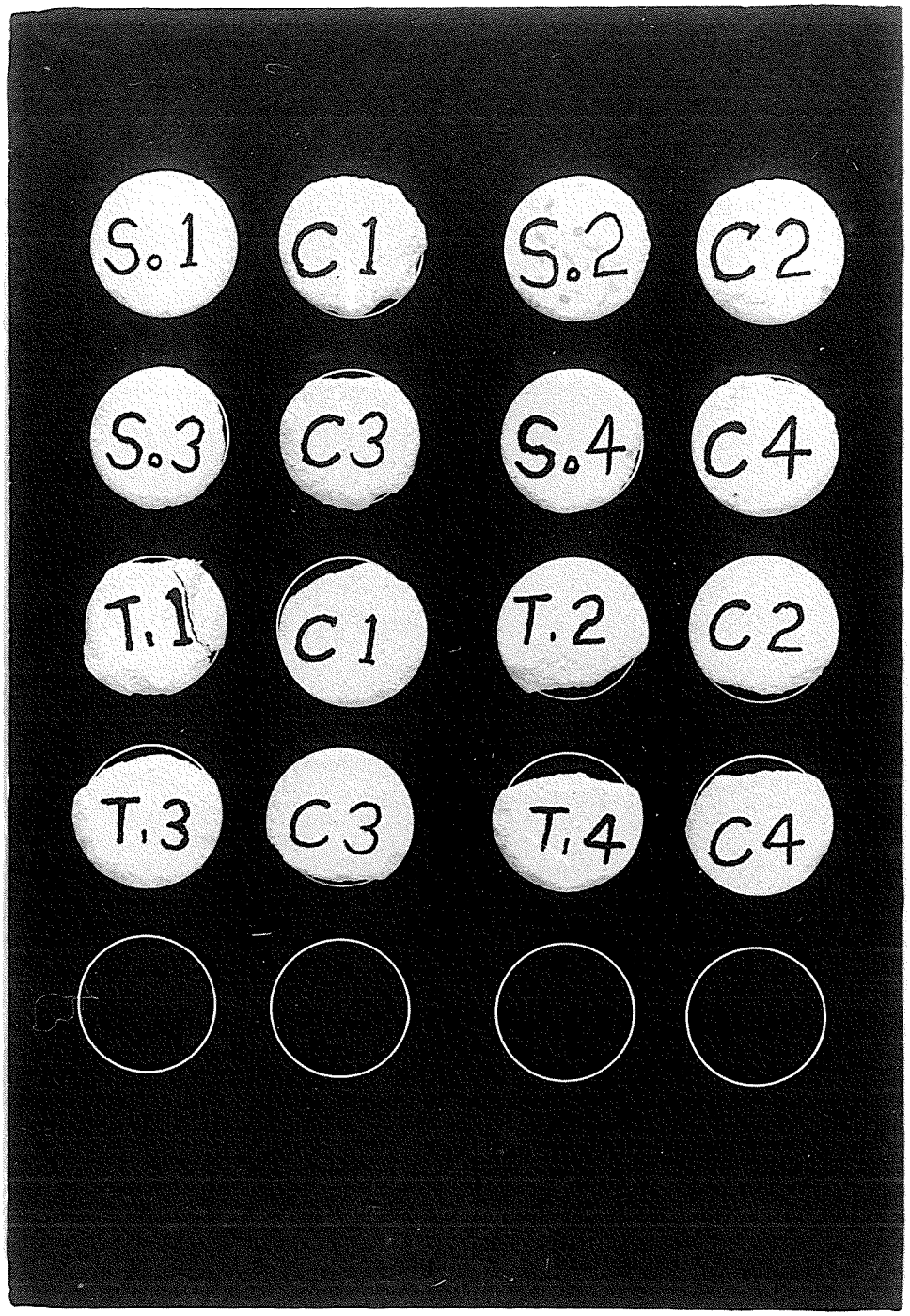

Fig. 40.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing either solanine (So) or tigogenin (Ti) was not significantly different to feeding on control wafers (C).

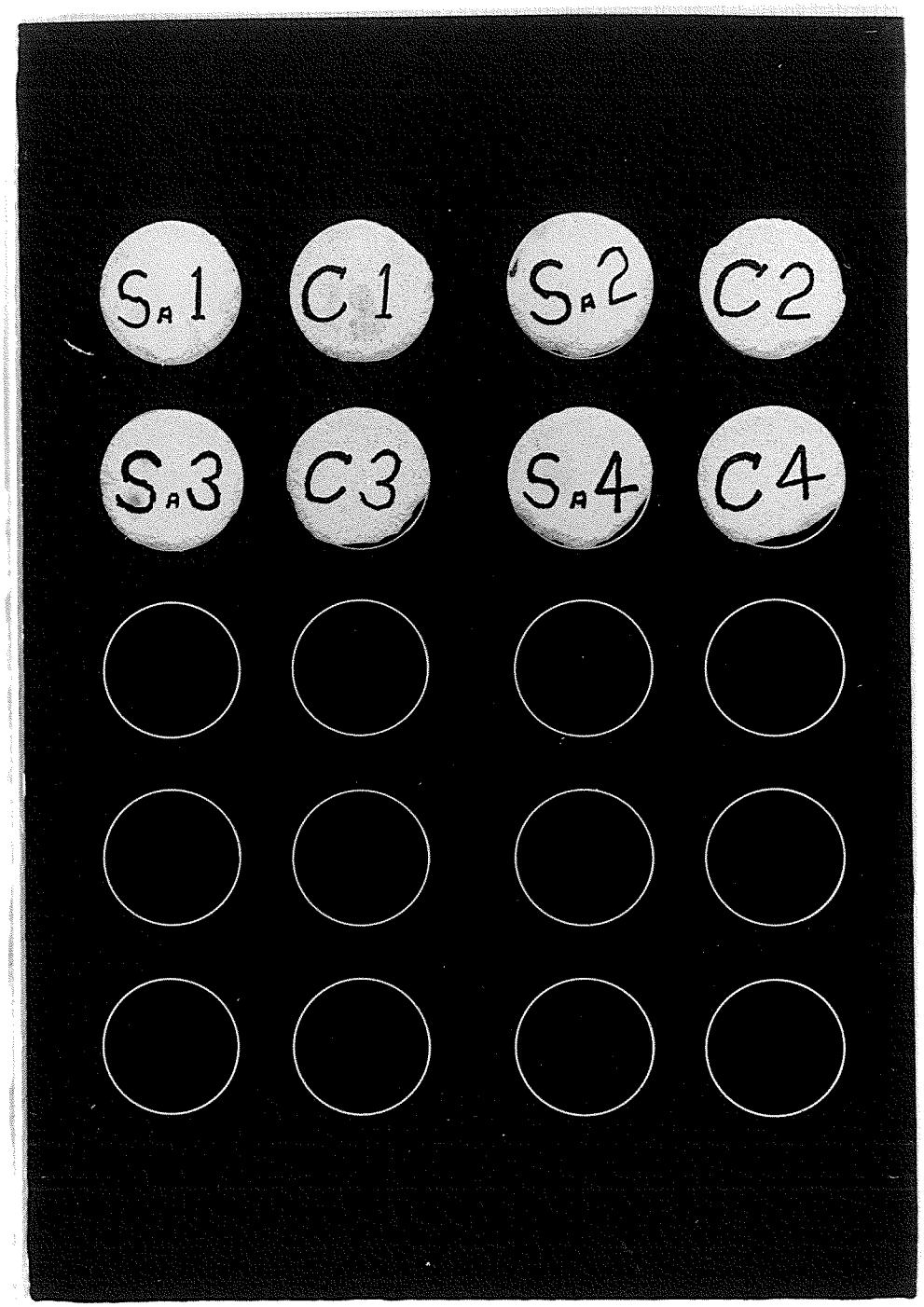

Fig. 41.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing santonine (Sa) was not significantly different to feeding on control wafers (C).

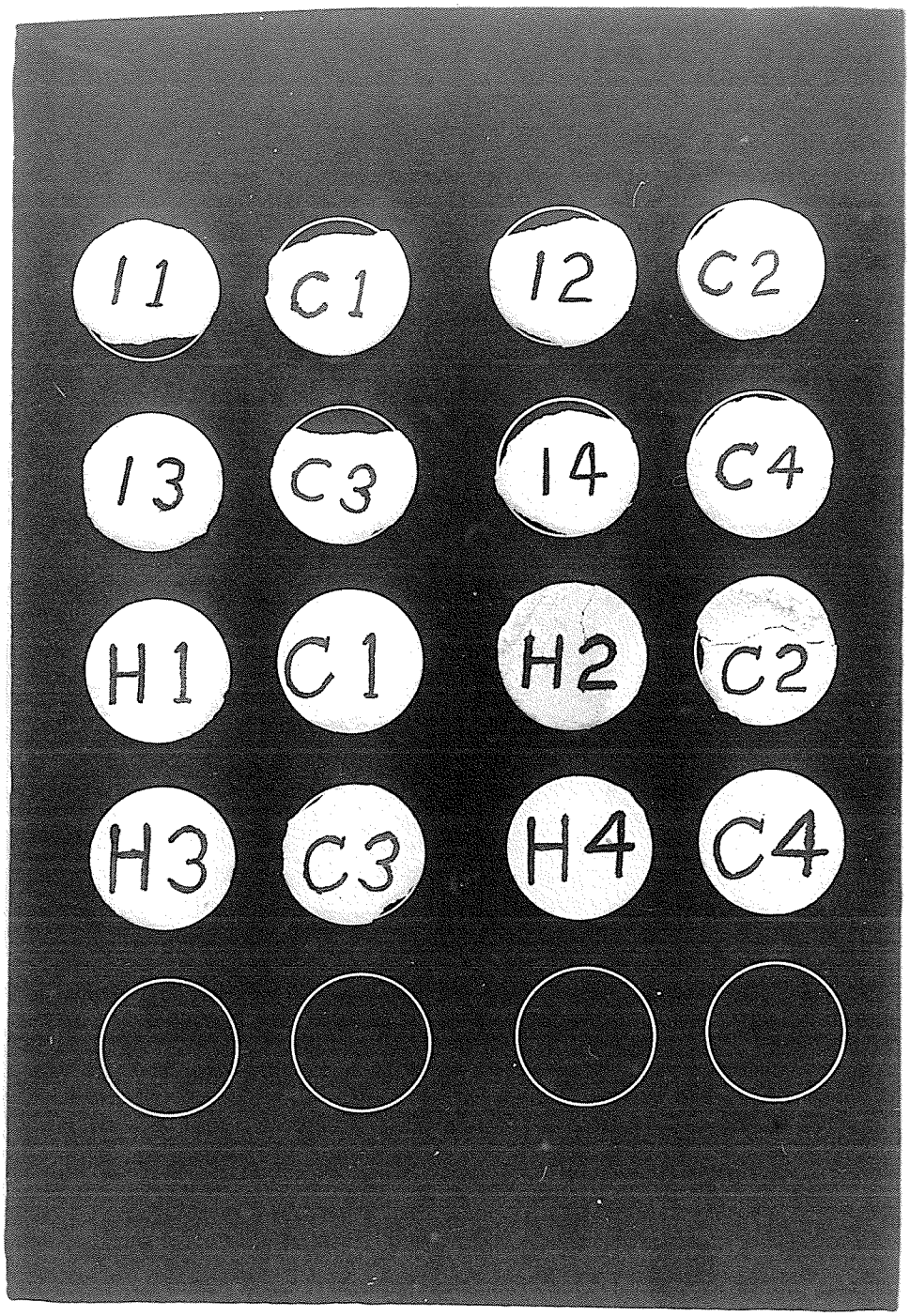

Fig. 42.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing either indican (I) or hydrastine hydrochloride (H) was not significantly different to feeding on control wafers (C).

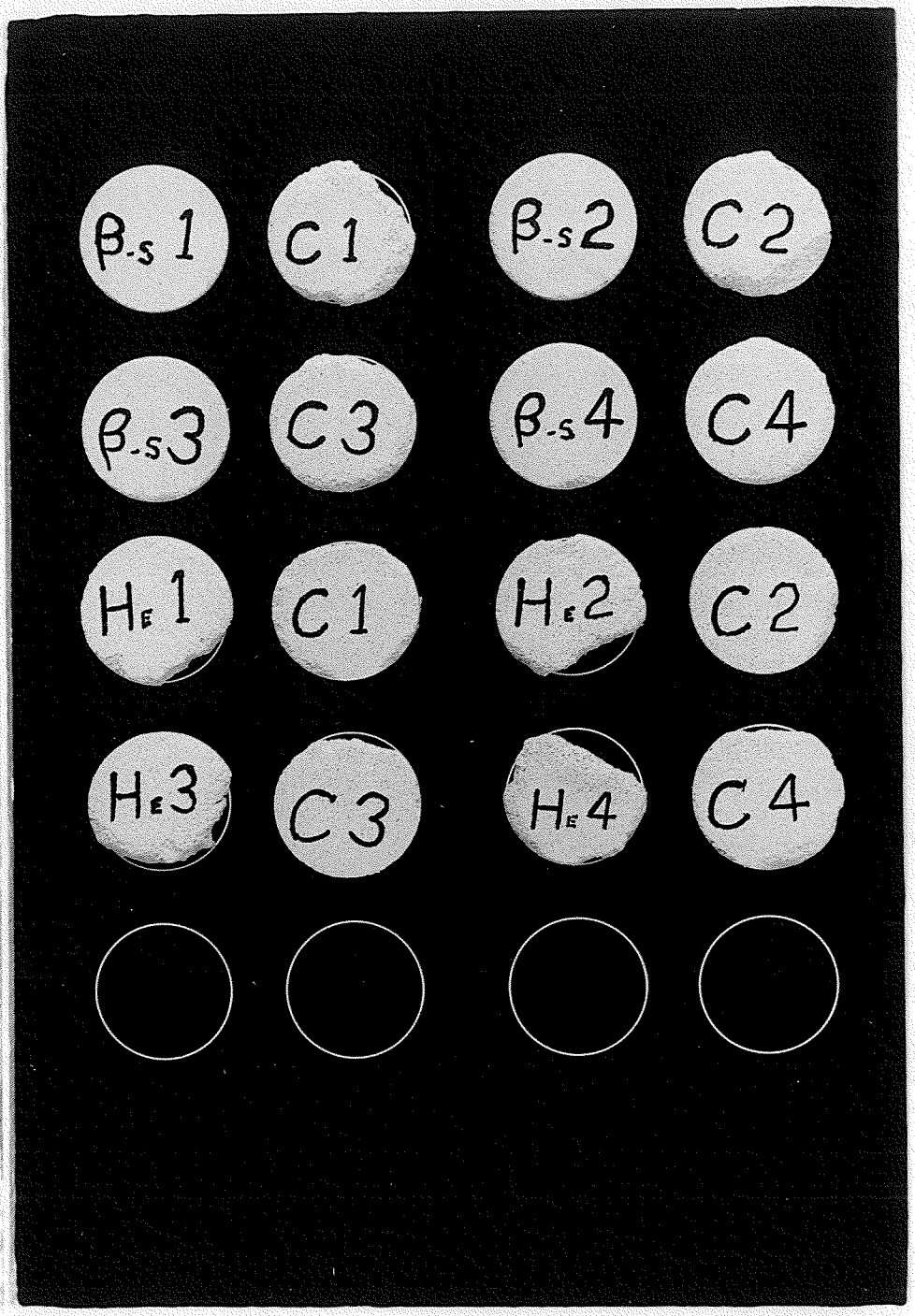

Fig. 43.- Portions of wafers not eaten in preference experiments. Feeding on wafers containing either β-sitosterol (βs) or hecogenin (He) was not significantly different to feeding on control wafers (C).

TABLE XVI

RESULTS OF FEEDING PREFERENCE EXPERIMENTS.
COMPARISON OF RELATIVE AMOUNTS OF FEEDING
ON TREATED AND CONTROL WAFERS

Chemicals with which feeding on treated wafers was not significantly different to feeding on control wafers	Chemicals with which feeding on treated wafers was significantly less than on control wafers	P value
Arbutin	Diosgenin	0.01
Hecogenin	Digitonin#	- x
Hydrastine hydrochloride	Gramine	0.025
Indican	Hordenine sulphate	0.025
Santonine	Hyoscyamine hydrochloride	0.025
Saponin#	Lobeline sulphate	0.005
Solanine#	Lupinine	0.005
β-sitosterol	Nornicotine dipicrate#	0.001
Stigmasterol	Veratrine	0.05
Tigogenin		
Tomatine#		

x data for statistical analysis were not available as the wafers disintegrated when placed in water.

in feeding experiments these chemicals were lethal.

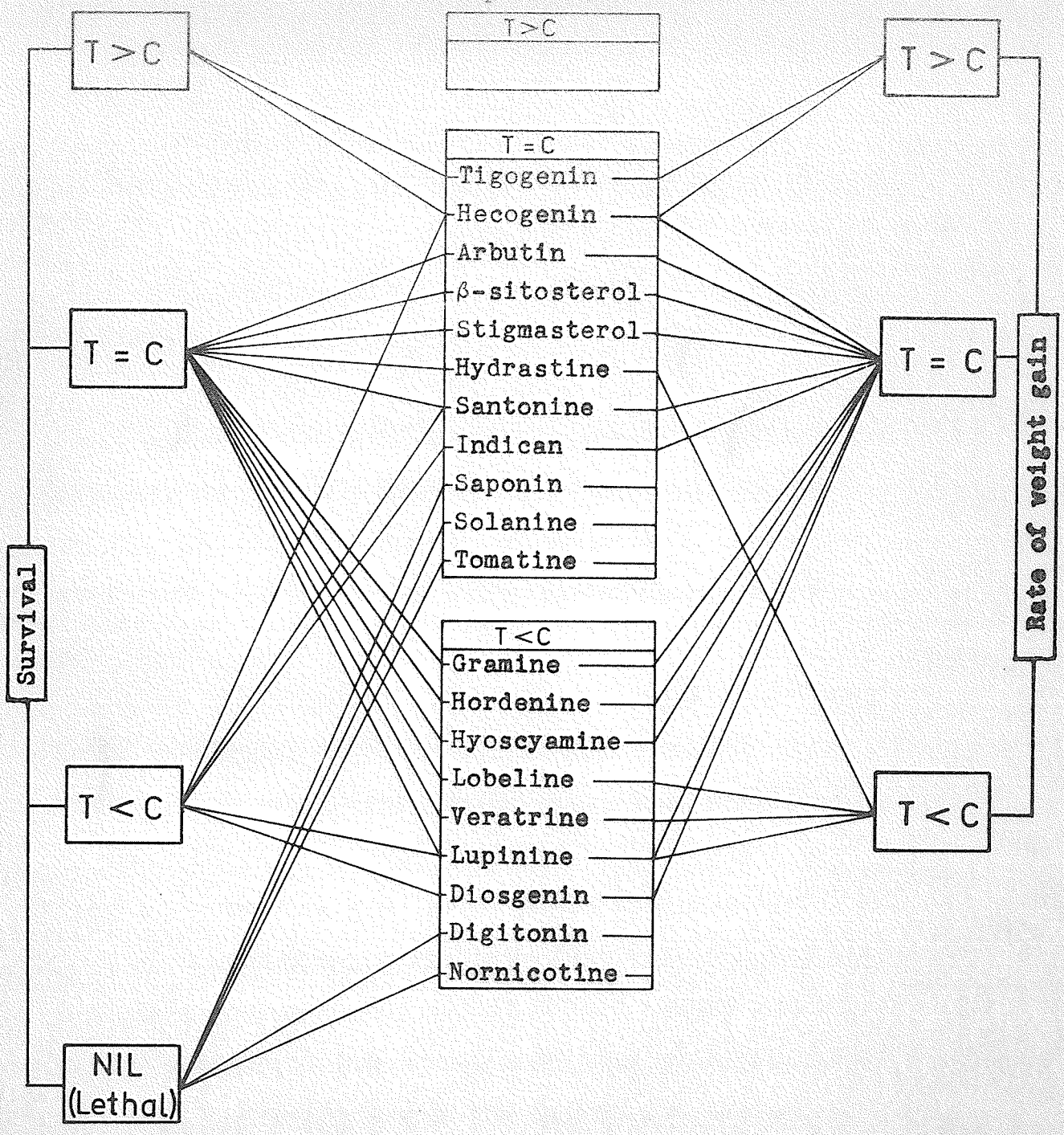

Fig. 44.- Comparison of effects of test chemicals on feeding behaviour (preference experiments), on survival, and on rate of weight gain (growth experiments).

T = diet to which test chemical was added.

C = control diet.

III. DISCUSSION OF EFFECTS OF TEST CHEMICALS ON DEVELOPMENT AND SURVIVAL RELATIVE TO FEEDING BEHAVIOUR

On the basis of feeding behaviour of M. bivittatus, test chemicals were divided into two approximately equal groups. The chemicals in one group did not affect feeding behaviour, whereas those in the second group reduced feeding.

Test chemicals in the group which did not affect feeding behaviour would not be expected, on teleological grounds, to affect survival. This expectation was fulfilled by six, and possibly seven test chemicals. However this group includes chemicals which reduced survival, (indican and possibly santonine), and which were lethal, (saponin, solanine, and tomatine) (Fig. 44). Unmitigated feeding by M. bivittatus on diets containing chemicals which reduce survival, or are lethal, would jeopardise continued survival of the species. However the species is a major pest which frequently attains high populations (Mitchener 1956). Lack of discrimination against diets containing these toxic chemicals indicates the insect either avoids feeding on plants containing them because of some concomitant repellent or inhibitory property, or throughout its evolutionary history the insect has encountered these plants infrequently and hence appropriate selection pressures have not been operative, and the insect has not developed the ability to discriminate against plants containing these toxins.

Two-thirds of test chemicals in the group which reduced feeding in preference experiments did not affect survival. Of these, two reduced rate of weight gain. Hence approximately half the test chemicals in this group were teleologically misplaced. By discriminating against diets containing these chemicals the insect was unnecessarily avoiding food sources which are nutritionally satisfactory and without toxic effects.

Data on the physiological effects of steroid chemicals on phytophagous insects were reviewed in a previous section. In summary, a particular sterol may itself fulfill completely the sterol requirements of an insect, or it may have a sparing effect on an essential sterol, or it may not fulfill any metabolic function. Many of the steroids investigated were toxic, some were lethal.

A wide range of steroids occur in plants in a great variety of combinations (Bergmann 1953, Heftman 1963). In view of the effects of chemicals in this group on survival and growth of insects it is reasonable to postulate a mechanism of insect-food plant association based on occurence of steroids in plants and their utilizability by, or toxicity to, insects. Data presented for M. bivittatus fed synthetic diet to which a range of these chemicals were added support this hypothesis.

These laboratory experiments support observations made in the field by various workers (see review of literature) that Melanoplus spp. feed on a number of plants, but not on all plants, within their geographic range.

It is conceivable that a method of combatting insect pests may be the development of varieties containing chemicals which, while innocuous to the consumer, will reduce feeding by insects. For example, if nymphs of M. bivittatus hatched in an extensive field of a barley variety high in hordenine or gramine, preferential feeding on weeds would be expected, however if the field was weed free, feeding on barley and normal growth by the insect may be expected. This example also illustrates the necessity of conducting not only behavioural experiments, for example, feeding preference experiments, but also experiments on metabolic effects of plant chemicals in order to gain an understanding of their true roles in feeding by phytophagous insects.

These data are not consistent with expectations of easy success in development of resistant varieties. However, a crop that is less palatable to grasshoppers than weed plants may possess an economically desirable attribute.

CHAPTER V

SUMMARY

In this study the effects of twenty chemicals of plant orign on the survival, development, and feeding behaviour of an insect, Melanoplus bivittatus (Say),which feeds on a botanically diverse range of plants were investigated. Some workers have postulated that plant chemicals, such as those employed in this investigation, are important in determining insect-host plant associations.

In growth experiments hatchlings were fed chemically defined synthetic diets to which test chemicals were added. Survival, rate of development, and weight of insects fed diets containing a test chemical, were compared with data for insects fed control diet.

Feeding behaviour was investigated in two series of experiments. In one series inhibition of drinking response by solutions or emulsions of a test chemical, when applied to the palps of adult grasshoppers, was regarded as an indication of the effect of the chemical on feeding behaviour of the insect.

In a second series of experiments feeding preferences of fifth-instar nymphs were investigated. Amounts of feeding on diets containing test chemicals were compared with amounts

of feeding on control diets. Relatively less feeding on treated diet was regarded as indicating that a chemical was a feeding inhibitor for M. bivittatus.

In growth experiments, the steroids, tigogenin and possibly hecogenin, were the only chemicals which increased survival. These chemicals increased rate of weight gain but did not affect adult weight.

Twelve chemicals, gramine, hordenine sulphate, hyoscyamine hydrochloride, lupinine, santonine, arbutin, digitonin (1%), β-sitosterol, stigmasterol, hydrastine hydrochloride, lobeline sulphate, and veratrine, had no effect on survival. Gramine and digitonin (1%) were the only chemicals which reduced adult weight. No chemicals increased adult weight. Hydrastine hydrochloride, lobeline sulphate, and veratrine were the only chemicals which reduced rate of weight gain.

Indican and diosgenin, and possibly santonine, hecogenin and lupinine, reduced survival. The effects of lupinine on adult weight and rate of weight gain were inconclusive. The other four chemicals had no effect on weight or overall rate of weight gain.

Five chemicals, nornicotine dipicrate, solanine, tomatine, digitonin (3%), and saponin, were lethal.

Results of drinking response and feeding preference experiments indicated that approximately half the test chemicals had no effect on feeding behaviour, and approximately half reduced feeding. None of the chemicals increased feeding.

Three chemicals, saponin, solanine, and tomatine, which were lethal in growth experiments did not affect feeding behaviour in preference experiments. Lack of discrimination against lethal chemicals, and against other less toxic chemicals, was teleologically unexpected.

Approximately one-quarter of the test chemicals did not affect survival or development, but reduced feeding in preference experiments. By discriminating against diets containing these chemicals the insect was unnecessarily avoiding food sources which are nutritionally satisfactory and without toxic effects.

All insects require a dietary source of a nutritionally adequate sterol. For phytophagous insects, this need must be supplied by food plants, Teleologically insect food plants should contain an adequate supply of a nutritionally satisfactory sterol but should not contain sterols or steroids toxic to insects. There exists a wide range of plant steroids and the steroid content of individual plant species varies considerably. Hence, probably only a restricted range of plants can supply the sterol requirements of a particular insect species. Other plants may not supply the insect's sterol requirements or may contain toxic steroids. The occurence of nutritionally adequate steroids, non-utilizable steroids, and toxic steroids has been postulated as a basis of insect-host plant associations.

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APPENDIX I

A LIST OF CHEMICALS RECORDED FROM PLANTS OCCURING WITHIN, OR CONGENERIC WITH PLANTS OCCURING WITHIN, THE GEOGRAPHIC RANGE OF *M. BIVITTATUS*

Chemical	Species from which the chemical has been recorded	Food plants of *M. bivittatus* or congeneric with food plants	References
ALKALOIDS:			
Gramine	*Hordeum jubatum*	+	7,8,9,2
	H. nodosum	+	3
	H. vulgare	+	
Hordenine	*Andropogon sorghum*	+	7,8,3,5
	Panicum miliaceum	+	7,9,8,3,5
	Hordeum murinum	+	7,9,8,3,5
	H. vulgare	+	7,9,8,3,2
Hydrastine	*Hydrastis canadensis**		2
Hyoscyamine	*Hyoscyamus albus*		3,2
	H. muticus		3,2
	H. reticulatus		3,2
	H. niger		3,4
	Datura quercifolia		3,2
	D. meteloides		3,2
	D. metel		3,2
	D. fastuosa		3,2
	D. arborea		3,2
	D. alba		3,2

Chemical	Species from which the chemical has been recorded	Food plants of *M. bivittatus* or congeneric with food plants	References
Hyoscyamine	*Datura stramonium*		3,2
Lobeline	*Lobelia urens*		3,5
	L. inflata		3,5,2
	L. syphilitica		3,5
	L. erinus		3,5
	L. cardinalis		3,5
	L. sessiliflora		3,2
Lupinine	*Lupinus luteus*		8,3,2
	L. niger		8,3,2
	L. palmeri		8,3,2
Nicotine	*Asclepias syriaca*	+	1,3,2
Nornicotine	*A. syriaca* ?	+	3,2
	Sedum acre		3,2
Santonine	*Artemisia maritima*	+	7,8,4
Solanine	*Solanum nigrum*	+	3,6,2
	S. dulcamara	+	3,6,5
	S. lycopersicum	+	6,2
	S. tuberosum	+	6,2,4
Tomatine	*S. lycopersicum*	+	4
Veratrine	*Schoenocaulon officinale*	*	4
GLUCOSIDES:			
Arburin	*Arctostaphylos uva-ursi*		3,4
	Vaccinum macrocarpon		3,4
	V. myrtillus		3,4

Chemical	Species from which the chemical has been recorded	Food plants of *M. bivittatus* or congeneric with food plants	References
Indican	*Polygonum tinctorum*	+	7,9,3,4
STEROLS:			
β-sitosterol	Widely distributed	+	4
Stigmasterol	Widely distributed	+	4
SAPONINS or SAPOGENINS:	Widely distributed. The following were selected as readily available representatives of the group.		
Digitonin	*Digitalis purpurea* *		4
Diosgenin	*Dioscorea* spp. *		4
Hecogenin	*Agave* spp. *		4
Saponin	*Quillaia saponaria* *		
Tigogenin	*Digitalis lanata* *		4

* indicates genera and species not recorded within the range of *M. bivittatus*.

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