Fecundity and oviposition behaviour of the cowpea seed beetle, *Callosobruchus maculatus* (Fabricius).

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by

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ABSTRACT

The reproductive biology of three strains of Callosobruchus maculatus F. (Coleoptera: Bruchidae), the cowpea-seed beetle, was studied. This beetle is a serious pest of stored legume seeds in the semi-arid tropics.

As females aged, energy reserves were depleted and aspects of this decline were related to the number of eggs laid in order to explain the observed daily egg laying pattern of C. maculatus. Various factors affected the fecundity of females. The initial adult weight of females showed a strong positive relationship with the number of eggs laid. Substances, which could be extracted from cowpeas, were shown to be necessary to allow normal oviposition on an artificial substrate, glass beads. The male contribution to female fecundity was also investigated.

Approximately half of the study was concerned with factors which govern a female's choice of oviposition site. The presence of a pheromone which enabled females to distribute their eggs more efficiently among cowpeas was demonstrated. This demonstration necessitated the development of a bioassay using a choice chamber which allowed beetles to choose between cowpeas marked with pheromone and control cowpeas. Using the bioassay, the solubility of the pheromone in different solvents was examined. The persistence of the pheromone over different periods of time was investigated and it was shown that the pheromone can remain active for at least thirty days.

In addition to the marking pheromone, the role of physical characteristics of the oviposition substrates was also studied. The surface area and weight of such substrates were shown to affect the choice of oviposition site by females.

The results obtained are discussed in the context of previous work on bruchids, particularly models of oviposition behaviour proposed by some workers.

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Chapter 1

INTRODUCTION

1.1 The importance of pulses as a protein source.

According to the Food and Agriculture Organisation, about 500 million people in developing countries suffered from chronic protein deficiency (Poleman, 1975) and with recent famines this figure is unlikely to have diminished. The production of animal protein is both expensive and inefficient in resource use when compared to vegetable protein. Because of this the provision of more vegetable protein is the only realistic way of meeting the demands made by a rapidly increasing population (Smartt, 1976).

The most important sources of food in the world are cereal crops but in many parts of the world, especially in developing countries, pulses (legumes) form the second most important source. Pulses contain about 20% protein (Centre for Overseas Pest Research, 1981) and in a diet which also contains cereals (as is usual) the two provide all the essential amino acids required to sustain life (Smartt, 1976). Thus food legumes are a valuable weapon in man's battle to feed himself.

Of the many species of food legumes the cowpea, Vigna unguiculata (L.) Walp., is one of the most important, especially in Africa where 91% of the

1.1 million tonnes per annum is grown (FAO production yearbook, 1975).

The factors limiting the expanded production and use of cowpeas include low yields (caused by poor soils and unfavourable climate), diseases and insect pests, and field shattering (where the pod dehisces before harvest) (Martin, 1984) but it is losses due to insect pests and moulds whilst in store that are the most serious of all in limiting the usable supply of cowpeas.

The importance of storage losses can be judged by a United Nations resolution passed in 1975 which called for improved food conservation to take priority over increased food production in developing countries (Harris & Lindblad, 1978).

1.2 Losses

Of the insect pests of the cowpea it is the Bruchidae which are the most important. All of the important pests of food legumes in store are members of this family (COPR, 1981), and they also damage crops whilst still in the field. Within the Bruchidae the major pests of the cowpea belong to the genus Callosobruchus of which two species, C. maculatus (F.), the cowpea seed beetle (sometimes known as the southern cowpea weevil), and C. chinensis (L.), the azuki bean beetle, are the most serious because of their widespread distribution and adaptability. C. maculatus is the most

common pest of stored legumes in Africa, whilst C. chinensis predominates in Asia (Southgate, 1964) both are cosmopolitan, however, being found in tropical climates throughout the world.

Three other pests in this genus are C. phaseoli, C. rhodesianus and C. analis. These also infest cowpeas although they may attack other legumes as well; C. phaseoli is also found on chickpeas, for instance. Two other important bruchid pests are Zabrotes subfasciatus and Acanthoscelides obtectus. Both originated in Central and South America but they are now cosmopolitan and are predominantly pests of Phaseolus species, especially P. vulgaris (indeed A. obtectus is rarely seen on any other seed - Southgate, 1978).

In Nigeria, the region of greatest cowpea production, large quantities of cowpeas are lost to bruchids every year (valued at £20 million per year - IITA, 1983). Infestation by C. maculatus may begin in the field whilst the pods are ripening. If the pods are picked as soon as they dry then damage to seeds may be limited (only 2.5% damaged) but if all the pods are harvested at the same time, so that some dry pods are left on the plant whilst others ripen, as many as 10% of the seeds may be damaged before they are placed in storage (Caswell, 1968).

It is in storage, however, that farmers suffer the greatest losses. Large scale concerns can usually afford to treat stored cowpeas with insecticides but such methods are very expensive and frequently beyond the scope of local farmers in developing countries.

Another factor which contributes to the level of C. maculatus infestation in cowpeas in developing countries is the value of the cowpeas themselves. This is because people, rather than destroying infested cowpeas, tolerate quite high levels of infestation (Dobie, pers. comm.) thus permitting the spread of the pest. Surveys of local markets in northern Nigeria showed signs of C. maculatus infestation in 50% of the seeds when cowpea stocks had been in storage for 3-4 months (Caswell, 1981).

1.3 Present control measures

Control measures dealing with bruchid infestation are available to wealthier farmers, such as those in California, which are simply beyond the scope of small scale farmers in developing countries. It is difficult to see how the "answer" to bruchid infestation, proposed by Taylor (1981), of commercial processing and fumigation combined with store hygiene, could be applied in developing countries at present.

However, there are relatively simple procedures which are easily followed and which will limit damage caused by pests and diseases. With many cowpea varieties, as already stated, it is desirable to harvest pods as they ripen since this reduces field, and subsequently storage, infestations by insects.

The cowpeas must always be dried to below 14% moisture content to prevent mould forming; large-scale farmers use mechanical dryers whereas those farmers who produce only small amounts of cowpea use the sun.

On small farms the cowpeas are usually stored in their pods since this offers some protection from beetles but even so the fraction of infested seeds can reach 32% (Caswell, 1968). Storage containers themselves offer varying degrees of protection from insect attack - mud and thatch granaries (called 'rumbus'), frequently used in Nigeria, would offer less than airtight metal bins, for example (COPR, 1981). Grain legumes are usually sold and transported in sacks; these do not prevent insects attacking seeds but if correctly stored in suitable warehouses with various refinements such as rodent-proof ventilation and fine-mesh screens on windows then losses can be kept to a minimum (although, again, these storage facilities are rarely available to small scale farmers).

Even under carefully controlled conditions infestations will occasionally occur and so pesticides must be used. It is also good practice to treat a storage area before a new batch of cowpeas are placed in it in order to remove residual infestations. Various insecticides (especially organophosphorus compounds) and fumigants, which are very successful in the close confines of a storage environment, can be used (COPR, 1981) although there is evidence of some degree of resistance to these compounds in bruchid populations (Evans, 1985). Again, it is recognised that such treatment may be beyond the scope of the small scale farmer.

Despite these control measures, the fact is that large amounts of stored cowpeas belonging to small-scale farmers remain relatively unprotected.

1.4 Resistant varieties of cowpeas.

It was to help such small scale farmers in developing countries and to combat resistance by beetles to insecticides that the Grain Legume Improvement Programme at the International Institute of Tropical Agriculture (IITA) near Ibadan, Nigeria screened cowpeas for resistance to attack by bruchids.

One variety of cowpea was found which significantly reduced the rate of increase of local populations of C. maculatus (IITA, 1981). The cowpea variety (known as TVu 2027) was later shown to be resistant because it contained a high level of a trypsin inhibitor which prevented the development to the adult stage of large numbers of larvae (Gatehouse et al, 1979). Although offering resistance to bruchids this variety gave a low yield and was highly susceptible to other diseases. TVu 2027 has been crossed with other varieties to give hybrid cultivars with better agronomic characteristics (IITA, 1983).

Although the programme to provide resistant varieties of cowpeas seems promising it would be foolhardy to introduce those varieties which have already been developed before the full implications of such an act are investigated. In an extensive study, Dick (1984) showed the importance of geographical variation among 'strains' of C. maculatus in their ability to develop on resistant varieties. Whilst TVu 2027 was resistant to the C. maculatus population local to IITA, it was not uniformly resistant to C. maculatus collected from other locations. In addition it appeared that those C. maculatus individuals which were able to develop on the resistant variety of cowpea passed this ability to their offspring, a greater proportion of which were able to develop on the resistant variety. This indicated that there would be a rapid evolution of the ability in some

C. maculatus populations to develop on the 'resistant' cultivars of cowpeas.

1.5 Geographical variation in C. maculatus.

For over fifteen years it has been known that 'strains' of C. maculatus collected from varying geographical locations had different biological characteristics (Fujii, 1968). Such differences are of immense practical importance when control measures are being devised for a specific pest - a control method which has been tested on one strain only may not be as successful when applied to other strains of the same species. This was clearly demonstrated by Dick (1984).

Dick worked on three different strains of C. maculatus and showed that differences between them included fecundity, oviposition behaviour, the time required for larval development, the number of individuals which would develop in a single cowpea and their ability to develop on TVu 2027. These strain differences are of great importance when the reproductive biology of this cosmopolitan species is studied because answers obtained for one strain do not necessarily hold for others.

1.6 Biology of *C. maculatus*.

In store the female beetle sticks its eggs directly onto the seed surface. On eclosion, after 5-7 days (depending on prevailing conditions), the first instar larva burrows directly through the part of the egg adjacent to the seed and the testa to form a feeding cell within the seed. The four instars feed and develop entirely within one seed and the cell is enlarged as the larva grows.

During the final instar the larva extends the cell until it is just below the surface of the bean leaving only the testa intact. At this stage the presence of a beetle within a cowpea can be detected by a small, round 'window' (the testa lying just above the cell) on the seed surface.

Once this pupal cell is formed just below the seed surface, the larva enters the pupal stage. This lasts around one or two days and is followed by adult eclosion, after which the adult chews through the testa around the edge of the window and emerges. The period of development from egg to adult varies with environmental conditions such as temperature; at 27 °C and 70% RH the period is about 30 days (Dick & Credland, 1984).

Emerging adults are usually between 1 and 5mm long (see Fig. 1.1), they are sexually mature and will copulate within minutes. Mated females may begin

Figure 1.1: Callosobruchus maculatus, the cowpea seed beetle.

The figure shows a female beetle at rest on a cowpea. The cowpea has several eggs attached to it.

ovipositing on the day of emergence and usually live for less than ten days. Adults do not normally feed under storage conditions but they may drink water and feed on pollen in the field (Alzouma, 1981).

C. maculatus is a species which is easy to maintain in the laboratory. Because of this, its relatively simple life-cycle and its importance as a pest it has been quite extensively studied. Despite this there are many significant details which are not known about the habits of C. maculatus (Southgate, 1981).

Many factors have already been identified which affect the fecundity of C. maculatus including the density of beetles (Brauer, 1945; Giga, 1982; Credland, Dick & Wright, 1986), host type and availability (Nwanze & Horber, 1976), humidity and temperature (Howe & Currie, 1964) and the provision of food for adult beetles (Larson & Fisher, 1938). Equally though, there remain other factors which warrant further investigation.

This includes one of the interstrain differences reported by Dick (1984) - the suppression of egg laying by females restricted to a few cowpeas (females on one cowpea laid significantly fewer eggs than those maintained for their adult life on forty cowpeas). Such suppression was evident in two strains but not in another.

The role of the host plant in triggering reproductive activity has been examined in several species of bruchids. Huignard (1979) reported that the host plant is a necessary stimulus to egg laying in most strains of A. obtectus; this was also found for Z. subfasciatus (Pimbert & Pierre, 1983). Ouedraogo and Huignard (1981) showed that the presence of the host plant is necessary to trigger egg laying in C. maculatus. Further investigation of the role of the host plant in the reproduction of C. maculatus was thought worthwhile. Of particular interest was the possibility of obtaining a host-plant extract which would trigger egg laying by female beetles in the absence of actual seeds as did Monge (1983) working on A. obtectus.

Mating and the role of the male in the reproductive activity of females has been studied in other bruchid species (Huignard, 1968, 1983; Pimbert & Pierre, 1983) but in C. maculatus it remains unclear whether repeated mating increases female fecundity. It is also unclear whether increased numbers of males cause increased egg mortality outside laboratory conditions; previous work having used exceptionally high adult densities (Utida, 1941a; Bellows, 1982a, b).

The issue of strain differences can be superimposed on all of these topics since the behaviour of one strain may not be the same as the behaviour of others. The basis for all of these investigations is discussed at

greater length in each chapter.

1.7 Oviposition and oviposition markers.

The immature stages of C. maculatus are spent entirely within the seed to which the egg was attached and so the larva cannot choose its own host - this is the responsibility of the parent female. In order to optimise its own fitness the female should, amongst other things, distribute its eggs in such a way as to reduce competition between larvae within the seeds. To do this a female should adopt an oviposition strategy which ensures that there are never too many larvae in a seed for the amount of food available.

A great deal of work has gone towards explaining the oviposition behaviour of different species of bruchids. C. chinensis has been studied the most and it has been stated that females of this species lay their eggs in a uniform manner, avoiding seeds with eggs already attached (Avidov, Applebaum & Berlinger, 1965; Avidov, Berlinger & Applebaum, 1965; Umeya, 1966; Nakamura, 1968). An oviposition strategy resulting in a uniform distribution of eggs has also been reported for A. obtectus (Umeya & Kato, 1970) (although Pouzat (1983) stated that egg laying of A. obtectus was random under certain conditions) and for C. maculatus (Gokhale & Srivistava, 1975). A uniform distribution of eggs might be the means by which a female maximises its fitness.

The optimum oviposition strategy for bruchids has been considered in detail by Smith & Lessells (1985) but perhaps the most straightforward hypothesis of oviposition strategy of a bruchid applies to C. maculatus (Mitchell, 1975). An essential element subtending this hypothesis (which will be discussed at length in later chapters), as with others, is that C. maculatus females must be able to recognise host seeds with eggs attached.

How might a female recognise eggs already laid? The physical presence of the eggs seems an obvious answer and this has been suggested by some workers (Messina & Renwick, 1985a) but there is also a great deal of evidence, applying to both C. maculatus and other bruchids, indicating that eggs are recognised, partly at least, by chemical markers (Yoshida, 1961; Oshima, Honda & Yamamoto, 1973; Giga & Smith, 1985; Szentesi, 1981).

In the case of C. chinensis, chemical substances are deposited by both sexes which, when applied to beans, deter subsequent oviposition - some active components of these substances have even been identified (Oshima et al, 1973). Substances which deter egg laying are also deposited by another bruchid pest, Acanthoscelides obtectus, (Szentesi, 1981), although the circumstances surrounding its function may be slightly different since the larvae, on emerging from the egg, do not simply bore into the nearest seed but may select a particular one from those nearby (Umeya & Kato, 1970). Although

circumstantial evidence has previously been presented for the existence of similar substances in C. maculatus (Mitchell, 1975), the first direct experimental evidence was published only recently (Giga & Smith, 1985).

These substances which deter oviposition are often known as 'oviposition markers' but the term 'oviposition deterring pheromones' is perhaps more accurate. Any pheromone which deters the oviposition of a pest species is of great interest. The possibility that the pheromone could be used as a control measure is an obvious channel for study - such a method has been used, in field trials, to control the European cherry fruit-fly (Rhagoletis cerasi) with some success (Katsoyannos & Boller, 1976).

It is also of interest, for both academic and practical reasons, to examine the biological properties of such a pheromone. In this way one may see how it is suited to its task of aiding in the efficient distribution of eggs by females and possibly establish some way of disrupting this process. In the study of the reproductive behaviour of insects and in the battle against this serious pest, the oviposition deterring pheromone of C. maculatus holds great promise.

1.8 Objectives of this study

The work described in this thesis forms part of a study into the factors which influence the reproductive biology of C. maculatus females. Of particular interest are those factors which influence the number of eggs which a female lays and those which influence its choice of oviposition site.

Chapter 2

MATERIALS AND METHODS

2.1. Culture of Animals

Three strains of C. maculatus were used; they are Campinas, Yemen and IITA. The Campinas strain was collected from Campinas, Brazil, in 1975 from cowpeas (V. unguiculata (L.) Walp.) and the Yemen strain was collected in 1977 from the Yemen Arab Republic from green lentils (Lens culinaris, Medik). The IITA strain was taken from an established culture kept on cowpeas at the International Institute of Tropical Agriculture, Nigeria in 1981.

The beetles were identified as C. maculatus by Dr. P. Dobie at the Tropical Development and Research Institute Laboratories, Slough and Mr. B. J. Southgate at the Ministry of Agriculture, Fisheries and Food Laboratories, Slough (Dick, 1984).

All three strains have since been cultured on dried cowpeas. These cowpeas, also known as blackeyes or black-eye peas, are grown in California and marketed by the California Bean Growers Association. Three varieties are sold - Cowpea #3, Cowpea #5 and Magnolia (the latter looks like a blackeye but is smaller in size); the former two are more common. There is an unknown mix in any bag (Fellows, pers. comm.).

During storage (before they were bought) these cowpeas were treated with either "Phostoxin" (Degesch Gmbh., Frankfurt) or methyl bromide. Neither treatment has any residual effect. After purchase cowpeas were kept frozen, at -20 °C, until they were used to prevent infestation by beetles and other pests.

To prepare a new culture of beetles, cowpeas were removed from the freezer and brought to room temperature. Using a sieve, adult beetles were separated from cowpeas of an existing culture and a number sucked into a pooter. These beetles were then placed with the fresh cowpeas in a jar which had a capacity of 3 litres. The inside of the necks of the jars were painted with "Fluon" (polytetrafluoroethylene (PTFE) dispersion) (ICI Ltd., London) which prevented beetles crawling up to the tops of the jars and so made handling of open jars much easier. The jar was sealed with a filter-paper disc which was held in place by paraffin wax. In these conditions adult beetles were able to mate and oviposit freely until death.

Soon after the cultures were established it was realised that a standard method of culturing should be adopted; this was to produce experimental animals which had developed under similar conditions. It was found during early experiments that the weights of females from different cultures were significantly different in some cases.

To standardise the cultures 400 adults (unsexed) were used to establish each new culture. A standard volume of cowpeas was also used each time the beetles were cultured. For the Campinas and IITA strains 600 ml of cowpeas were used; for the Yemen strain 800 ml were used. Because fewer Yemen adults emerge from a single cowpea (Dick & Credland, 1984) more cowpeas were used for cultures of this strain; this allowed the collection of sufficient numbers of adults for experiments.

Fresh subcultures were established weekly so that at any time five subcultures of each strain were maintained. One had been established for a month and was at the peak emergence of adults. Another was a week older than this and contained adults which had emerged after the peak emergence. The other three cultures were one to three weeks old and contained no emerged adults but had larvae developing inside the cowpeas. The adults used to establish the culture always died before the next generation emerged so that the two generations never mixed.

Only one generation of adults was collected from any culture jar. Old cultures usually became mouldy and so they were destroyed by freezing after the fifth week to prevent interference with other cultures.

Since several cultures of each strain were kept this could also mean that cultures of the same strain could become genetically isolated from each other. To prevent this adults from chronologically adjacent cultures were mixed together to establish a new culture each week. For the Campinas and IITA strains 300 adults were taken from the culture which was at peak emergence that week and 100 adults were taken from the culture which was at peak emergence during the previous week (this culture was then destroyed). For the Yemen strain 200 beetles were taken from each culture because of this strain's slightly longer development period (Dick & Credland, 1984).

Mark (1982) stated that as a result of different feeding regimens C. maculatus develops different rates of oviposition over several generations. He said that the beetles tended to time their oviposition so that emergence of the next generation coincided with collection for the next culture. Emerging females would delay oviposition until they were placed onto fresh beans of a new culture. The culturing method adopted here goes some way to diffuse such selection pressure as the time at which beetles may emerge and still be selected for the next culture is extended across the normal period of emergence (Dick & Credland, 1984).

Cultures were kept in a constant temperature and humidity (CTH) room; this was maintained at 27±1 °C and 70±10% relative humidity with a 12 hour photoperiod.

This temperature and humidity is close to the optimum conditions for the development and reproductive activity of C. maculatus (Larson & Fisher, 1938; El-Sawaf, 1956; Howe & Currie, 1964; Giga & Smith, 1983). Panji & Gill (1974) state that adults of C. maculatus copulate and oviposit with equal efficiency both in darkness and light.

Four cultures were sampled to find the average density of larvae penetrating individual cowpeas. In no case did the density exceed 8 larvae per cowpea (means were 6.78, 7.82, 7.9 and 7.22 with n = 50 and s. error = 0.5 for all four values). Adults emerging from cowpeas at different larval densities have been shown to have differences in fecundity which are statistically significant. However, the actual differences are unlikely to be of any great practical significance (Credland, Dick & Wright, 1986).

C. maculatus is described as showing polymorphism, having an "active" or "flight" form as well as a "normal" or "flightless" form (Caswell, 1960; Utida, 1965,1968; Sano, 1967; Taylor & Agbaje, 1974; Nwanze & Horber, 1975), although the normal form is able to fly (Messina & Renwick, 1985b and pers. obs.). The "flight" form is rarely found in laboratory cultures kept at low densities. This is possibly because of competition between the two forms; the flight form has a later oviposition period and produces fewer eggs (Utida, 1981).

The flight form was not seen in the cultures used for this study.

2.2. Sieving and Conditioning Cowpeas

To produce experimental conditions which were as standard as possible, within practical limits, the cowpeas used in experiments were graded for size. Small cowpeas were removed using a sieve with circular holes 6.7 mm in diameter. No attempt was made to remove large cowpeas but few exceeded 12 mm in length.

Cowpeas were then spread in a thin layer, covered with gauze or muslin, and placed in the CTH room. This was done to stabilise the temperature and moisture content of the cowpeas. The cowpeas were conditioned in this way for several days.

Since only egg laying was studied and not the subsequent development of the larvae, it was felt that cowpea moisture content was not so critical as temperature. Studies of this beetle have shown that temperature is far more important than humidity (unless at extremes) in influencing the reproductive activity of C. maculatus (El-Sawaf, 1956; Giga & Smith, 1983). Since cowpea moisture content is determined to a large extent by humidity one must suppose that it too has no major influence on the activity of the adult beetle.

2.3. Experimental Equipment

In all experiments beetles were isolated either in glass tubes or in plastic Petri dishes. The glass tubes were 2.5 cm in diameter by 7.5 cm high; the Petri dishes were 8.5 cm in diameter by 1.3 cm deep.

Experiments in which beetles were isolated for more than 24 hours were run using glass tubes. As females sometimes lay eggs on glass the tubes were lined with emery cloth (Grade No. 80, English Abrasives Ltd.) which prevented the beetles laying eggs anywhere but on the cowpeas. The tubes were closed with foam stoppers which were permeable to air.

The Petri dishes were not lined with emery cloth. Where beetles were isolated for only 24 hours oviposition on the containers was rare. To allow air to circulate a hole (2 cm in diameter) was cut into the lid; this was covered by plastic gauze which was held in place by plastic cement ("Tensol" Cement No. 6, ICI Ltd., London).

Both tubes and Petri dishes were easily washed in detergent to remove any traces left by the beetles and the emery cloth liners were rinsed in alcohol.

2.4. Collecting Beetles for Experiments

Adult beetles were collected from the cultures. Cowpeas were taken from the culture jar in which adults were at peak emergence. These were first sieved to remove adults which had already emerged. The cowpeas were then spread in a single layer in several Petri dishes. In this way adults could be collected very soon after they emerged, usually within five minutes. Collection took place in the CTH room and where possible, because of variation between cultures, beetles for the same experiment were collected from a single culture on the same day.

Newly emerged beetles were placed together in an empty Petri dish and any pairs which copulated were isolated. There was no previous opportunity for mating and copulation usually began within a few minutes of emergence. If mating did not seem to have been properly completed (sometimes females will kick off a male soon after mating has begun) these pairs were discarded. Usually however copulation lasted three to five minutes.

To obtain virgin females beetles were simply isolated on emergence before they had an opportunity to mate.

2.5. Weighing of Beetles

Where it was necessary to weigh females they were lightly anaesthetised using CO_2 gas and weighed on a Sartorius Microbalance 4501 giving an accuracy of ±0.001 mg. Nwanze & Horber (1975) record no ill effects in *C. maculatus* after a short exposure to CO_2.

2.6. Killing Beetles

Beetles required for dissection were killed using ethyl acetate vapour and then frozen.

2.7. Dissection of the Reproductive System

Beetles were kept frozen until just before dissection to minimise deterioration of the tissues. The dissections took place in a beetle Ringer (for composition see Ramsay, 1964, also given in Appendix 1), this reduced distortions of the tissue due to osmotic effects. There was no recognisable difference between the tissues of comparable beetles which had been dissected whilst fresh and those which were frozen.

2.8. Counting Eggs

Eggs were counted at least seven days after they were laid. This allowed the eggs to hatch and the larvae to penetrate the testa of the cowpea. The frass produced by the larvae turns the eggs white and makes them more

easy to see. To count eggs laid over the entire lifetime of a particular female the cowpeas were left for eighteen days after the female was placed onto them. This time period allowed for the life-span of the female, not more than 10 days under these conditions, and for hatching of the last eggs laid, four to seven days (El-Sawaf, 1956).

2.9. Statistical Analysis of Results

Differences in the mean egg laying totals of females from different experimental groups were analysed using a single factor analysis of variance with a-priori tests to analyse the difference between specific groups. The method for this test was taken from Sokal & Rohlf (1973). Analysis of variance is a fairly robust test which operates well, even with considerable heterogeneity of variances, and is affected only slightly by sizeable deviations from normality (Zar, 1974).

In order to quantify the distribution of eggs on cowpeas, so that comparisons could be made, a technique described by Iwao (1968) was used. In this technique the index of distribution is the ratio of mean crowding to the mean. In the present study, the mean refers to the mean number of eggs per cowpea and the mean crowding refers to the mean number of other eggs per egg on the same cowpea.

Mean crowding was calculated using the following formula -

$$\overset{*}{x} = \overline{x} + \left(\frac{s^2}{\overline{x}} - 1 \right) .$$

Where,

$\overset{*}{x}$ = mean crowding,

$\overline{x}$ = mean number of eggs per cowpea,

s^2 = variance,

(Lloyd, 1967).

It was not suitable to use an analysis of variance for comparisons of some sets of data so non-parametric tests were used. Such data included percentage hatching of eggs and the ratio of mean crowding to the mean. In these cases the Mann-Whitney U-test was used. Where sets of data were paired, such as the egg laying of females on a choice of two groups of cowpeas, the Wilcoxon matched-pairs signed-ranks test was used. These tests required less rigid assumptions about the data tested and were also very easy to apply.

Asterisks are used in most tables of results to indicate statistical significance. The number of asterisks denotes the significance level -

$* = p < 0.05$

$** = p < 0.01$

$*** = p < 0.001$

Values which were not statistically significant are indicated by N/S.

Chapter 3

FECUNDITY AND SUPPRESSION OF EGG LAYING

3.1. Introduction

Descriptions of the fecundity, defined as the number of eggs laid, of Callosobruchus maculatus are varied and this is not surprising when the number of factors involved are considered. Among the factors which are known to influence fecundity are the density of beetles, both in adult and larval stages (Brauer, 1945; Giga, 1982; Credland, Dick & Wright, 1986), host type and availability (Nwanze & Horber, 1976), humidity and temperature (Howe & Currie, 1964), and the provision of food for adult beetles (Larson & Fisher, 1938).

Since a positive relationship between adult weight and fecundity has been demonstrated in other laboratory insect species (Snyman, 1949; Ullyett, 1950), an experiment was conducted to see if there was a relationship between the weight of females at emergence and the number of eggs they laid.

Egg laying of C. maculatus, which does not feed under storage conditions (Dobie, 1981), is likely to be a major cause of energy expenditure and, hence, weight loss. So the weight loss of females was investigated in another experiment.

Daily egg laying was also recorded. It was thought that this might give an indication of the change in energy reserves of the females with both age and egg laying. The results obtained allowed egg laying to be related to weight loss.

Ahmed, Salem & Elbadry, (1976a) made a detailed study of the reproductive systems of C. maculatus but this did not include changes that occured as the females aged. By examining the reproductive systems of females of known ages and life histories any changes might be detected and related to other factors such as egg laying.

Differences in reproductive activity between strains (Dick & Credland, 1984) may have been due to relatively straightforward differences in the morphology of the reproductive systems. Variations in the ovariole number between strains could be responsible for differences in fecundity and have been described for other species (David & Bocquet, 1975; Suzuki & Yamada, 1976).

The daily egg laying patterns were also examined for comparison with the results of other workers using different strains.

One of the interstrain differences reported by Dick & Credland (1984) was that the IITA strain did not suppress its egg laying on a limited number of cowpeas whereas the Campinas and Yemen strains did. An experiment was carried out to further investigate this

difference by studying egg production and changes in the reproductive systems, as well as egg laying, of suppressed females.

Bruchids in storage environments do not always have a readily available supply of host seeds on hand when they emerge as adults. The reaction of females to such a situation was investigated by delaying the onset of oviposition.

3.2. Materials and methods

3.2.1. Daily egg laying, weight loss with egg laying, and change in oocyte number with age.

Mated pairs of each strain were collected and the females weighed. Each pair was placed in a lined glass tube, which contained 40 conditioned cowpeas and transferred to the CTH room.

Sets of replicates for each strain were killed after different lengths of time (as shown in Table 3.1) and the females weighed immediately. Two virgin females were also isolated with each group and were placed, separately, onto 40 cowpeas. They were killed and weighed in the same way as the mated females.

Where a pair or virgin were allowed to remain on cowpeas for longer than one day the cowpeas were replaced with fresh cowpeas every twenty-four hours. Replacing the cowpeas prevented any suppression of egg laying,

Table 3.1: Numbers of replicates of each strain and the time spent on cowpeas before death.

Time on Beans	Numbers of Replicates		
	IITA	Campinas	Yemen
1 Hour	10	--	--
2 Hours	10	10	10
4 Hours	10	10	--
1 Day	--	10	8
2 Days	10	--	--
3 Days	9	--	--
4 Days	8	10	10
5 Days	9	--	2
6 Days	8	1	--
7 Days	9	6	4

caused by lack of oviposition sites, and also allowed daily egg laying to be recorded.

Thus, for each female, weight at mating, weight at death and the number of eggs laid each day was known.

Some of the females were dissected to examine their reproductive systems. The number of ovarioles in each of the two ovaries was counted and then the number of discernible oocytes in each female. These were generally greater than 0.1 mm in diameter and were easily recognisable as oocytes (by their position in relation to the germarium) at x40 magnification. The lateral oviducts were then opened and the number of eggs present were counted. The contents of the bursa copulatrix and the vagina were then examined and any recognisable eggs counted. To avoid ambiguity a distinction is drawn between "oocytes" located within the ovarioles and "eggs" found in the lateral oviducts after ovulation. Egg production, as opposed to egg laying, is defined as the number of eggs laid plus those stored in the lateral oviducts and bursa copulatrix.

3.2.2. Emergence weight and fecundity

30 mated pairs of each strain were collected and the females weighed immediately after mating. Each pair was then placed on 40 fresh cowpeas and the female was allowed to oviposit until death. 40 cowpeas are enough to prevent suppression of egg laying by one female

(Dick & Credland, 1984).

3.2.3. 6-day egg laying

40 Campinas pairs were collected and placed in separate tubes containing forty conditioned cowpeas. In half of these females were allowed to oviposit, undisturbed until death. For the other 20 replicates the forty cowpeas were changed after 6 days for another forty, fresh cowpeas and the females then allowed to oviposit until death.

3.2.4. Suppression of egg laying

Reducing the number of oviposition sites.

60 mated pairs of each strain were collected; half of these were placed on two cowpeas only and the other half were placed on forty cowpeas. Beetles from 10 tubes of each of the two treatments were removed and killed after 6 hours. A further 10 were killed after 2 days and 6 days. 10 extra replicates were collected for the Campinas strain at five days.

The females were all dissected so that the oocytes and the eggs stored in the lateral oviducts could be counted; the number of eggs laid by each female was counted and the number of those which had hatched was noted.

Delaying the onset of oviposition.

20 newly emerged and mated Campinas females (isolated from their mates) and 20 virgin Campinas females were collected. These were kept separately in empty glass tubes with emery liners so that they were unable to lay eggs.

After 6 days each of the mated females was placed on forty cowpeas and allowed to oviposit until death. The virgins were allowed to mate with newly emerged males, separated, and each female placed on forty cowpeas to oviposit until death. For practical reasons any virgins which did not mate within a few minutes were discarded. This left twelve replicates from the initial twenty.

After 18 days the eggs were counted and egg mortality was noted. These values were compared with those of 30 Campinas females collected from the same culture which were allowed to mate and oviposit in the usual way.

3.3. Results

3.3.1. Daily egg laying patterns

The number of eggs laid daily by females of each strain are shown in Fig. 3.1. In each case most eggs were laid on the first day, followed by a continuous decline.

Figure 3.1: Daily egg laying by individual females of three strains of *Callosobruchus maculatus*

a) Campinas strain

Values given are mean daily totals (± 1 S.E.) laid by individual females on 40 cowpeas which were changed daily.

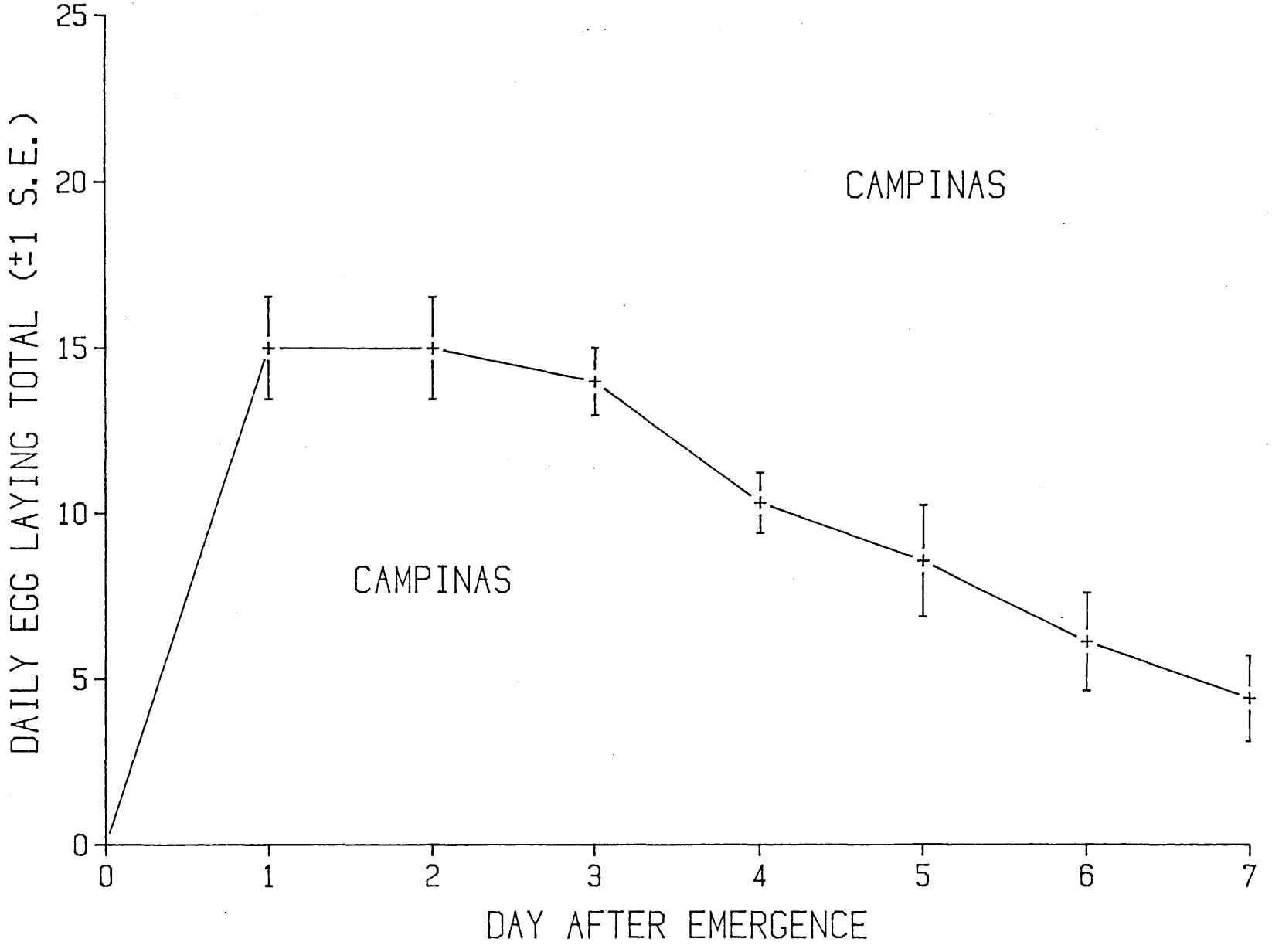

DAILY EGG LAYING TOTAL (±1 S.E.)
25
20
15
10
5
0
CAMPINAS
CAMPINAS
0
1
2
3
4
5
6
7
DAY AFTER EMERGENCE

Figure 3.1: b) IITA strain

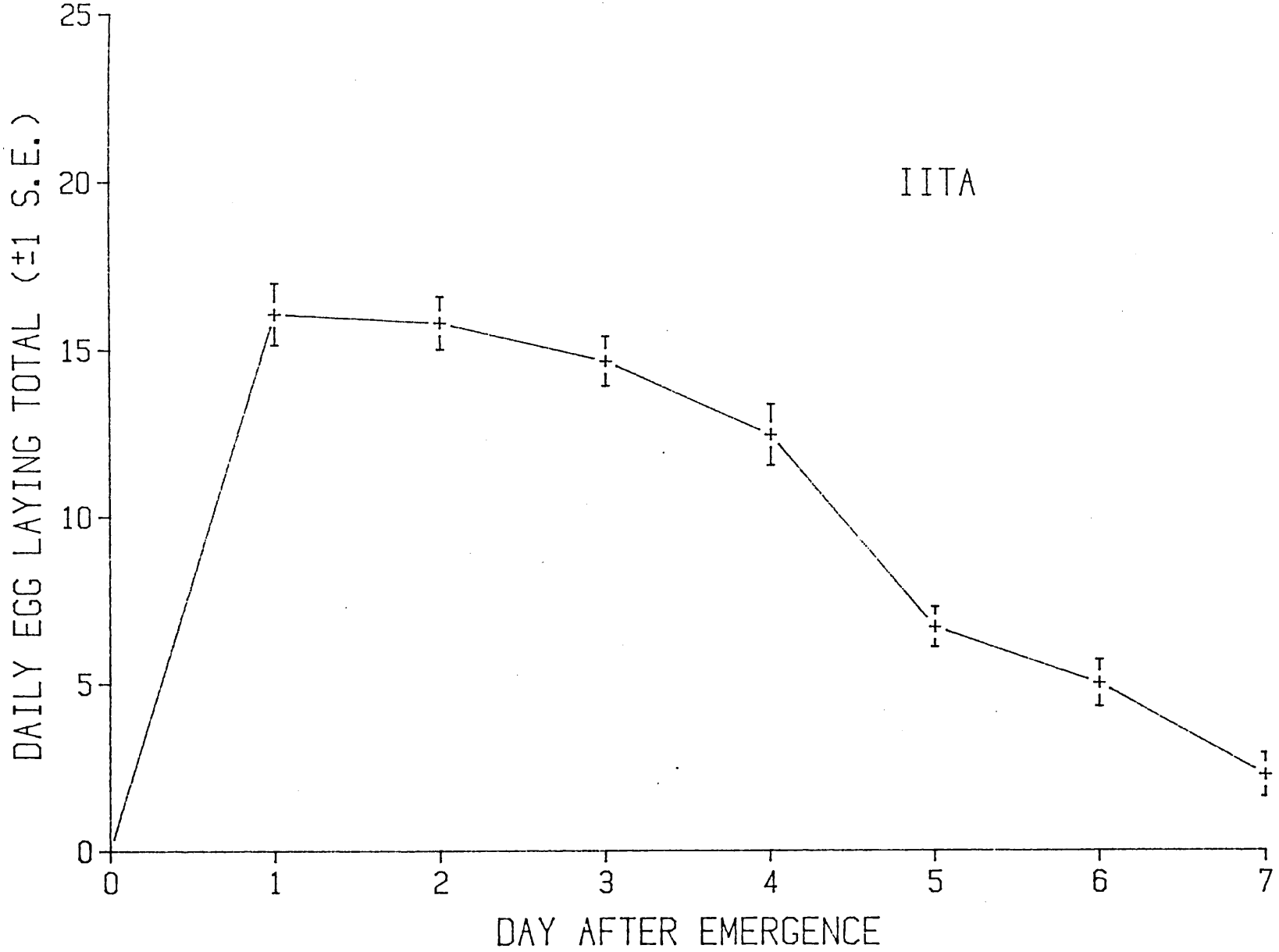
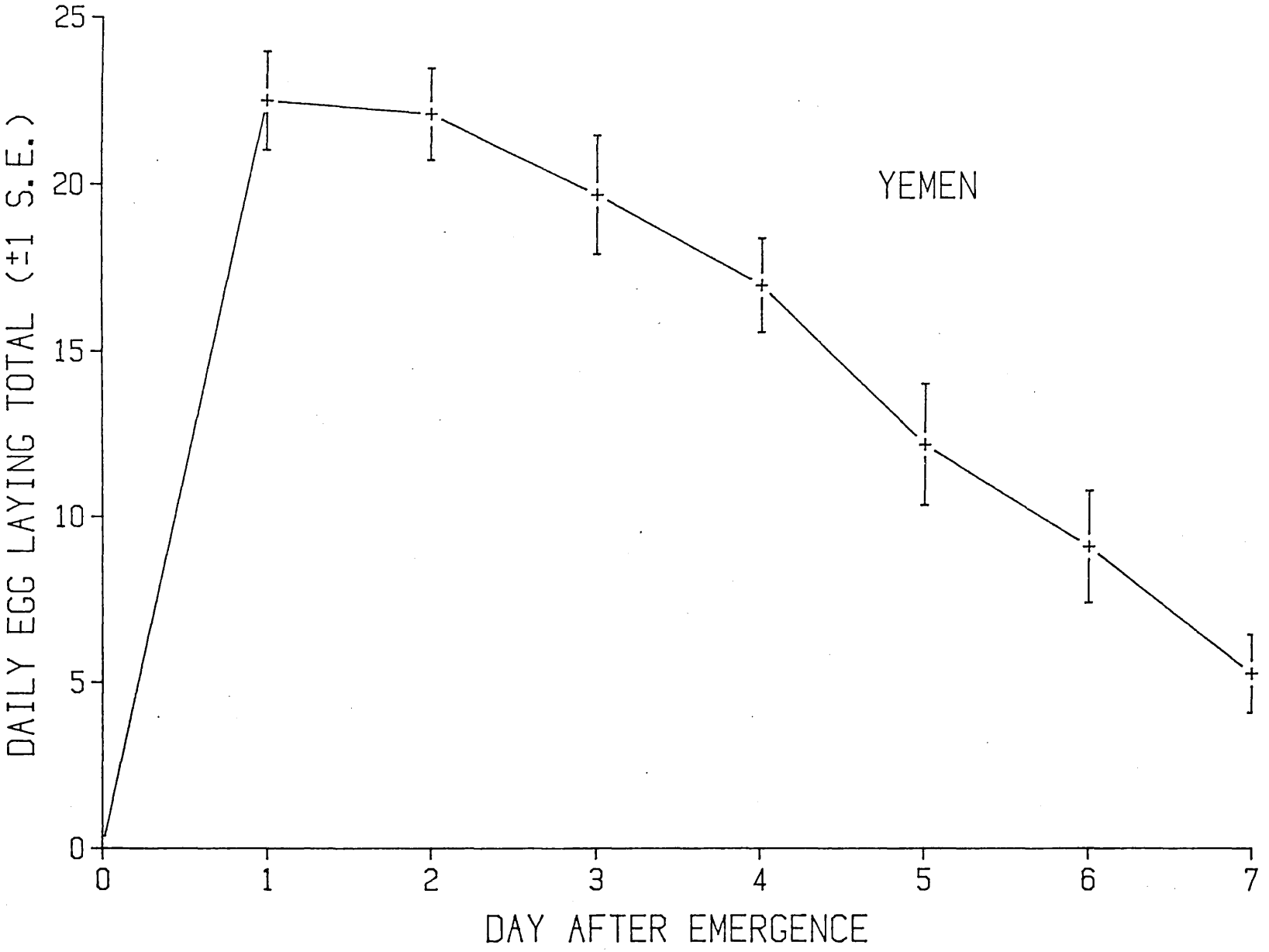

IITA
DAILY EGG LAYING TOTAL (±1 S.E.)
25
20
15
10
5
0
0
1
2
3
4
5
6
7
DAY AFTER EMERGENCE

Figure 3.1: c) Yemen strain


DAILY EGG LAYING TOTAL (±1 S.E.)
25
20
15
10
5
0
YEMEN
0
1
2
3
4
5
6
7
DAY AFTER EMERGENCE

3.3.2. Change in oocyte number with age

Figs. 3.2 and 3.3 show the reproductive system of a Campinas female. The reproductive systems of 305 females, of the three strains, were dissected and the number of ovarioles in each was noted. Only four females varied from the normal arrangement of 6 ovarioles in each ovary. All four were Yemen females; three had an extra ovariole in one ovary (13 ovarioles) and the fourth had an extra ovariole in each ovary (14 ovarioles).

The relationship between the average numbers of oocytes per female and age is shown in Fig. 3.4. For each strain a regression analysis was performed on the results for females which were between one and seven days old. The regression coefficients are presented in Table 3.2.

3.3.3. Weight loss and egg laying

The relationship between weight loss and egg laying is shown in Fig. 3.5. The regression coefficients are given in Table 3.3. Results for an analysis of covariance are given in Table 3.4.

Table 3.5 shows the difference in weight loss of 7-day old virgin and ovipositing females. The results include females of all three strains.

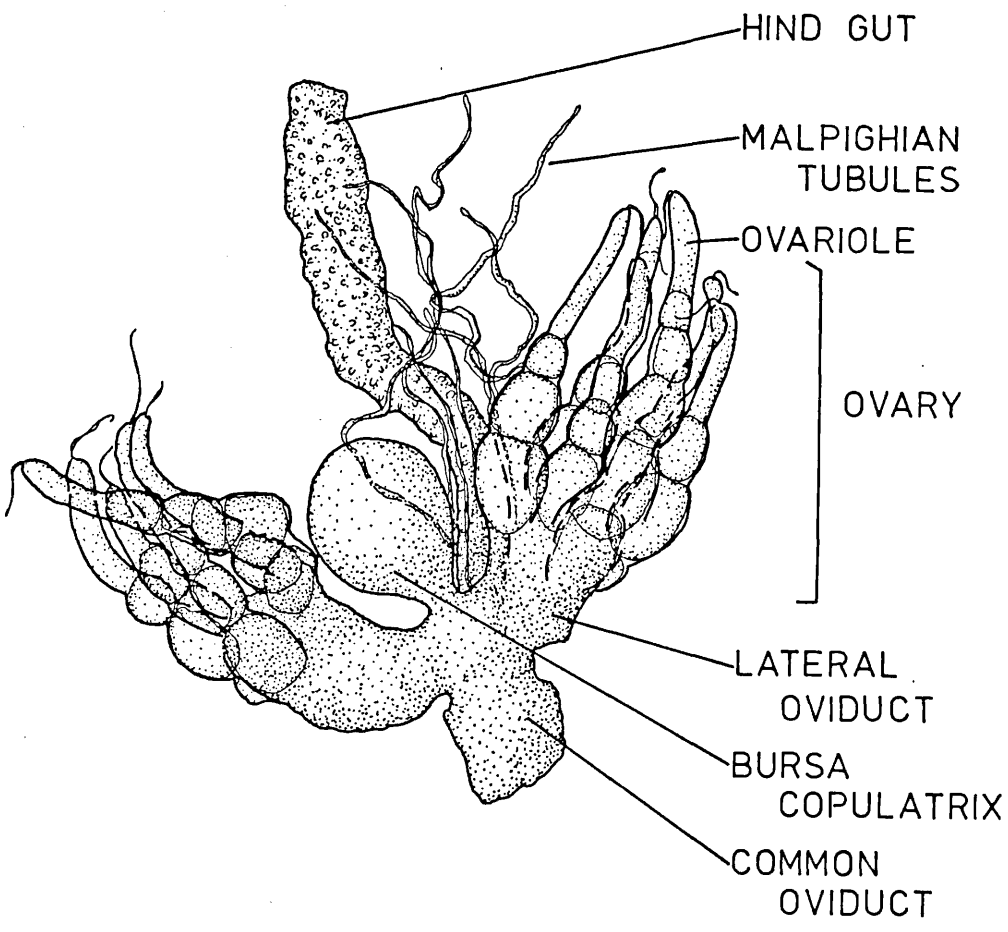


Figure 3.2: The female reproductive system.

Drawn from a Campinas female; magnification approximately x 35.

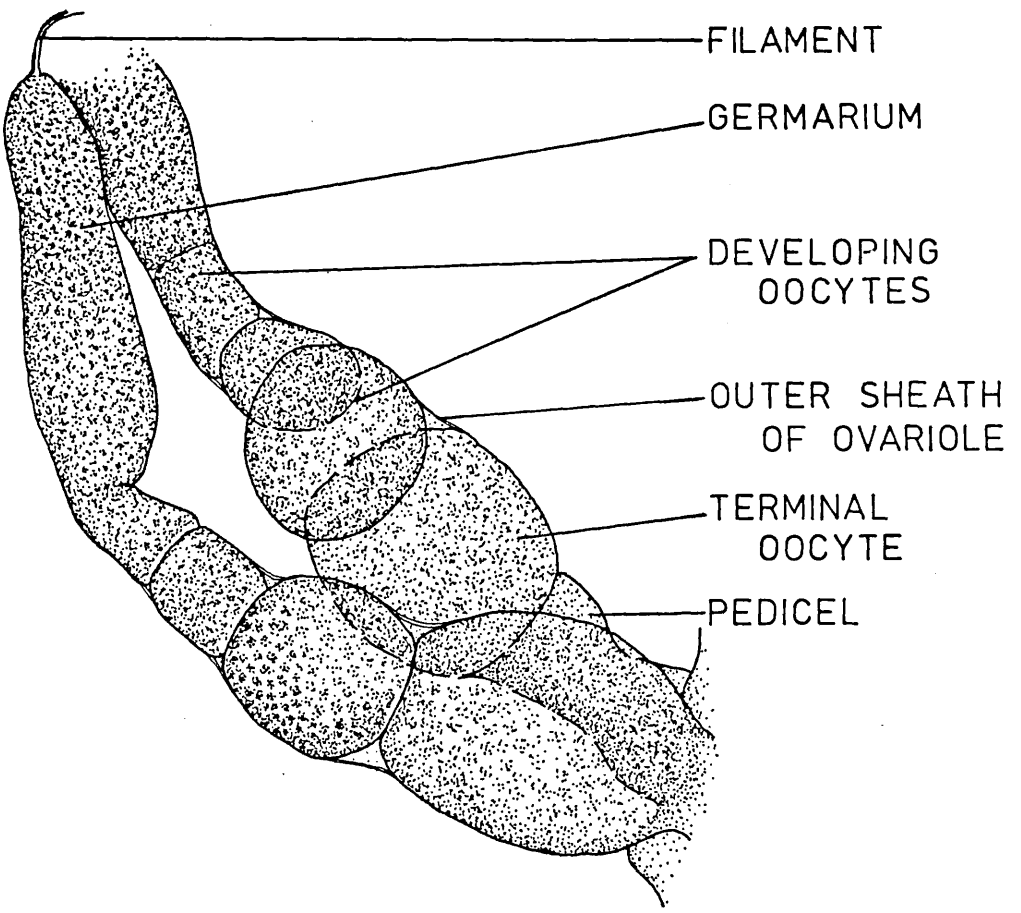


Figure 3.3: Close-up of an ovariole.

Drawn from a Campinas female; magnification approximately x 90.

Figure 3.4: Change in oocyte number with age.

a) Campinas strain

The values given are the total number of oocytes (generally greater than 0.1 mm in size) in both ovaries of individual C. maculatus females which were allowed to mate and oviposit freely. Regression coefficients for the lines of best fit are given in Table 3.2, all are significantly different from zero.

\+ - 1 observation

o - 2 observations

□ - 3 observations

* - mean value for all females of the same age.

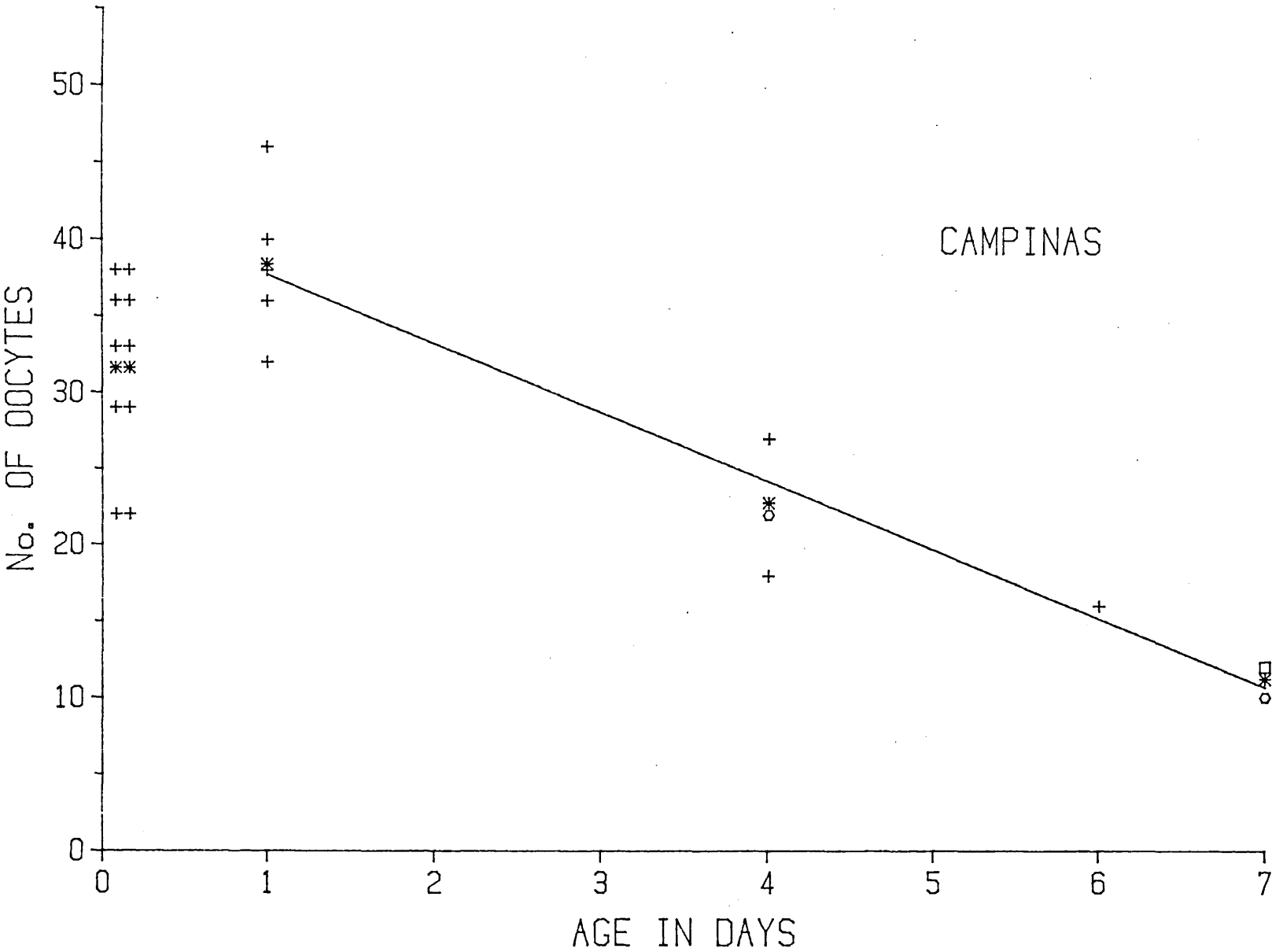

CAMPINAS
No. OF OOCYTES
AGE IN DAYS
0
10
20
30
40
50
0
1
2
3
4
5
6
7

Figure 3.4 b) IITA strain

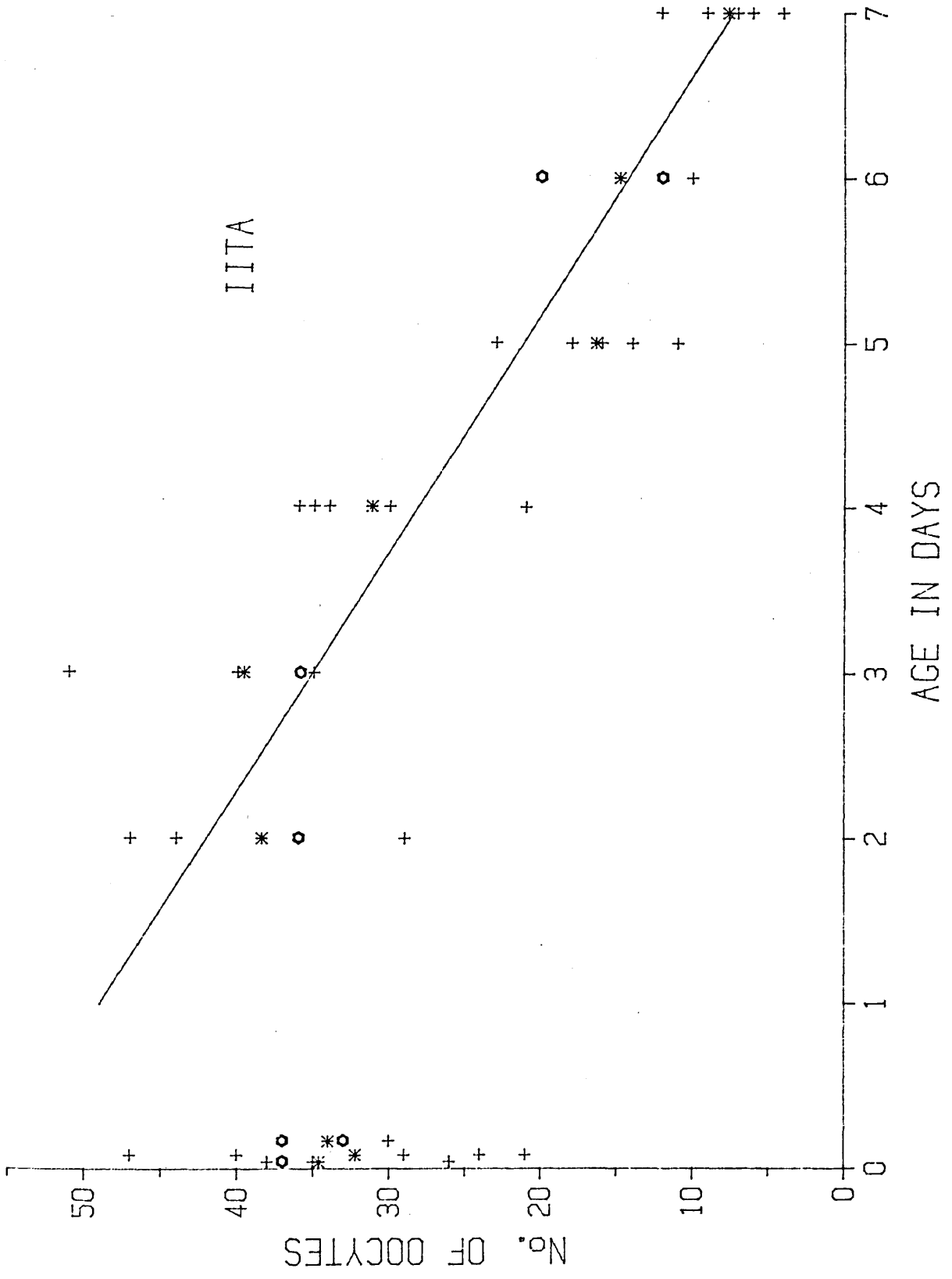

IITA
No. OF OOCYTES
AGE IN DAYS
0
10
20
30
40
50
1
2
3
4
5
6
7

Figure 3.4 c) Yemen strain

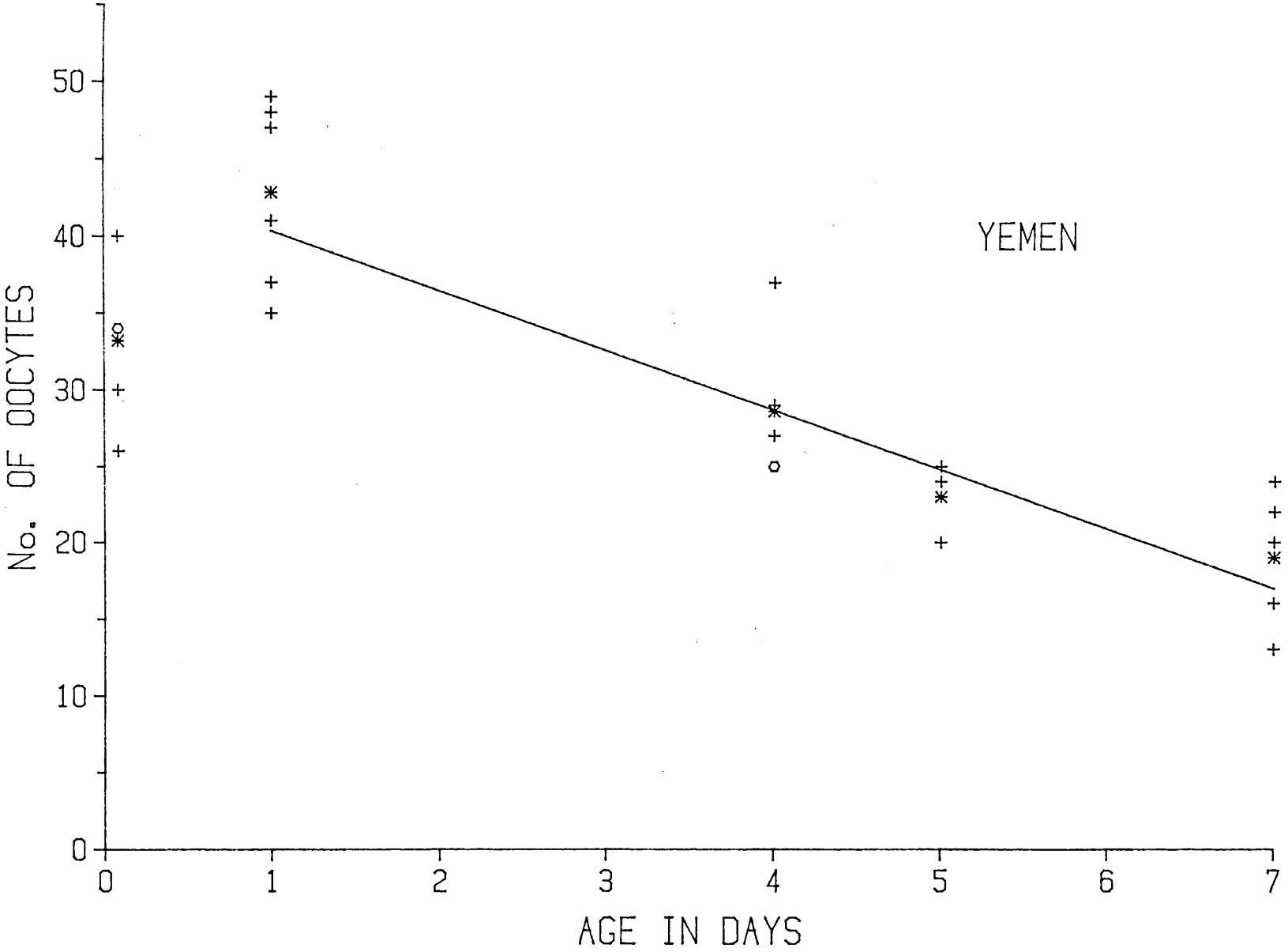

YEMEN
No. OF OOCYTES
AGE IN DAYS
0
10
20
30
40
50
0
1
2
3
4
5
6
7

Table 3.2: Regression equations for change in oocyte number with age.

Campinas:	Oocyte number	=	42.25	-	4.51	x	Age in days
IITA :	" "	=	55.94	-	6.95	x	" " "
Yemen :	" "	=	44.24	-	3.89	x	" " "

All regression coefficients are significantly different from zero ($p < 0.001$).

Figure 3.5: The relationship between egg laying and weight loss.

a) Campinas strain

The weight loss (mg) of individual C. maculatus females is plotted against the number of eggs which they laid. Regression coefficients for the lines of best fit are presented in Table 3.3, all are significantly different from zero. Analysis of covariance for the three strains is presented in Table 3.4.

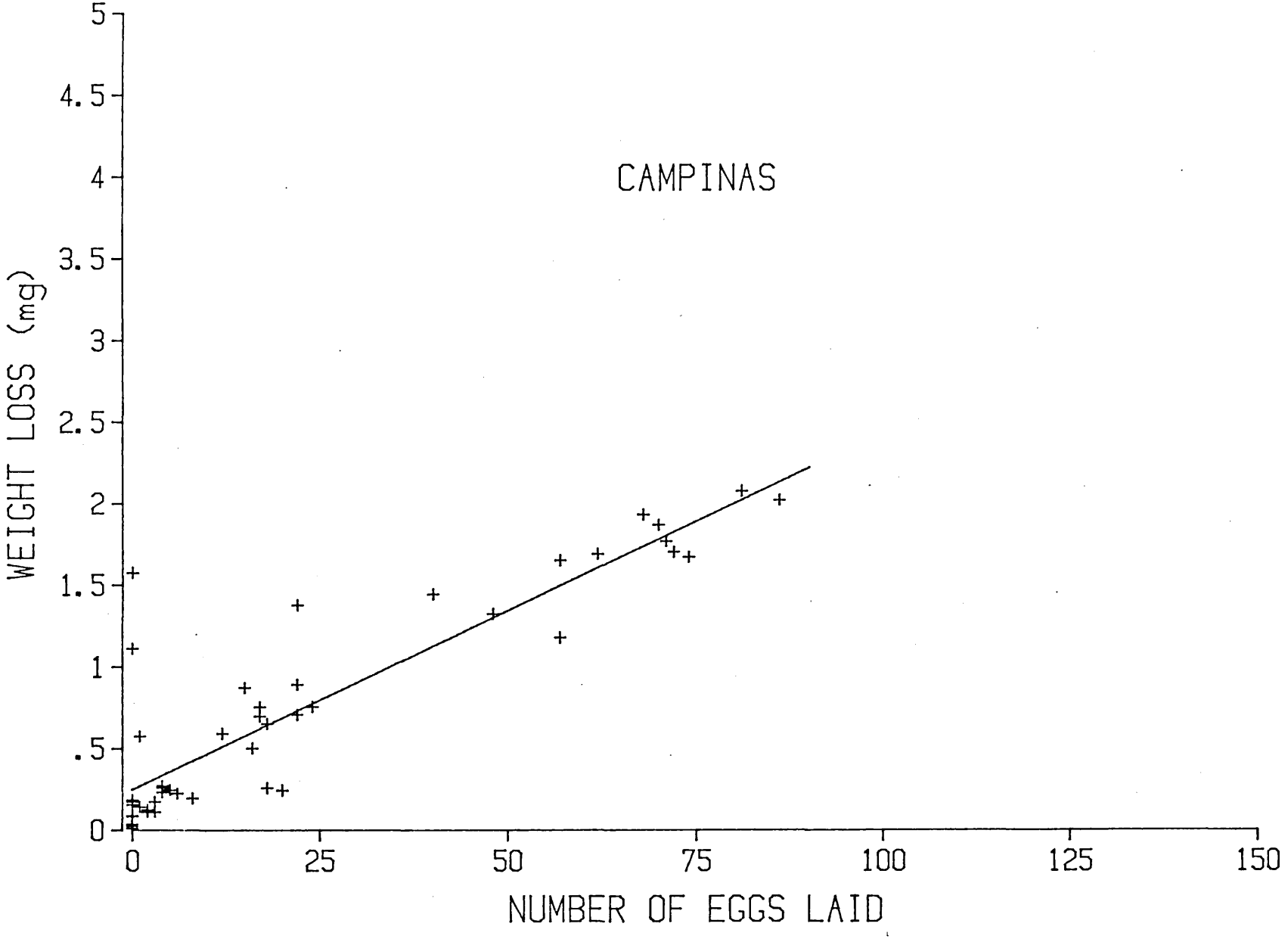

CAMPINAS
WEIGHT LOSS (mg)
NUMBER OF EGGS LAID
0
.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
25
50
75
100
125
150

Figure 3.5: b) IITA strain

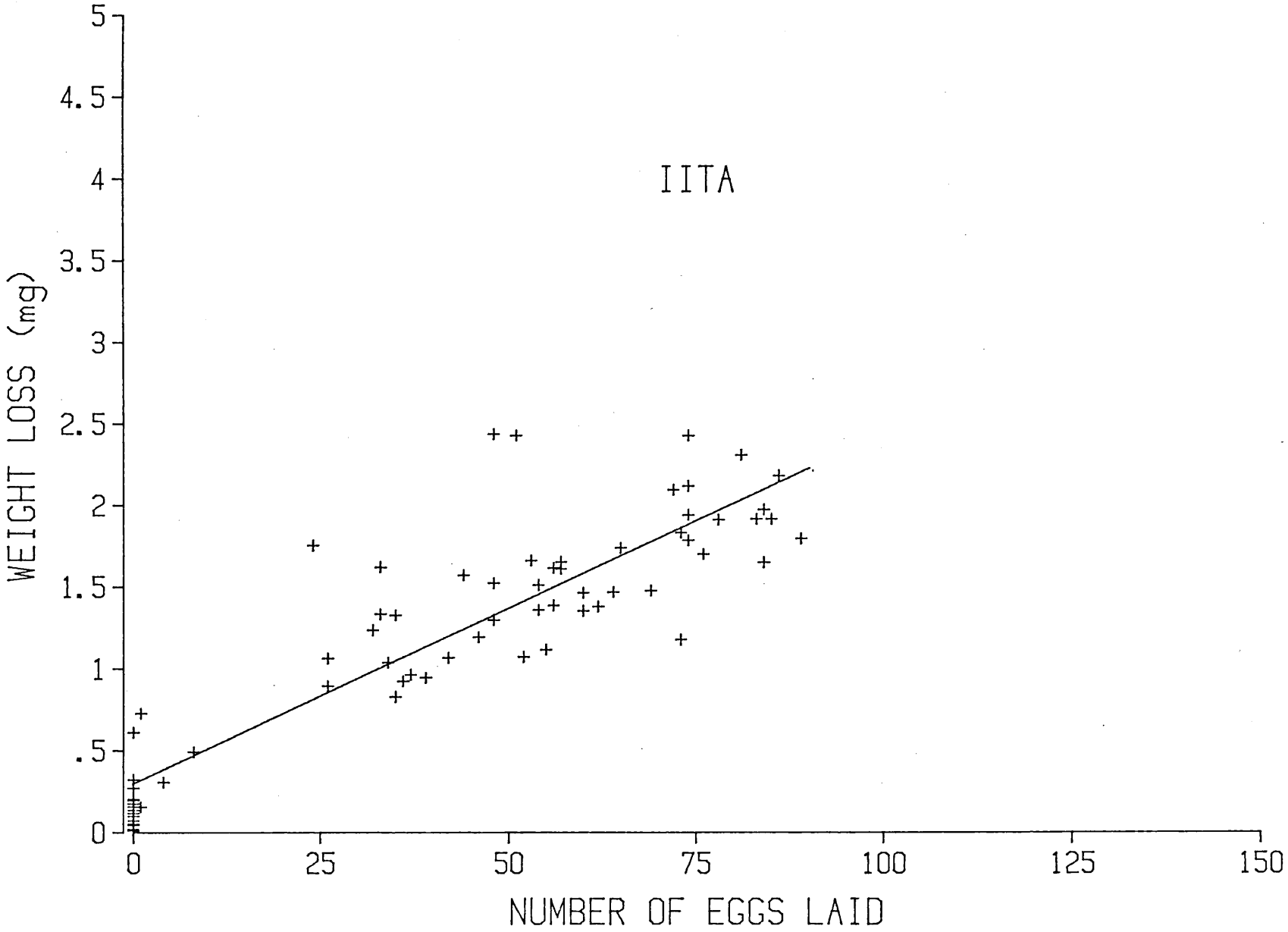

IITA
WEIGHT LOSS (mg)
NUMBER OF EGGS LAID
0
.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
25
50
75
100
125
150

Figure 3.5: c) Yemen strain

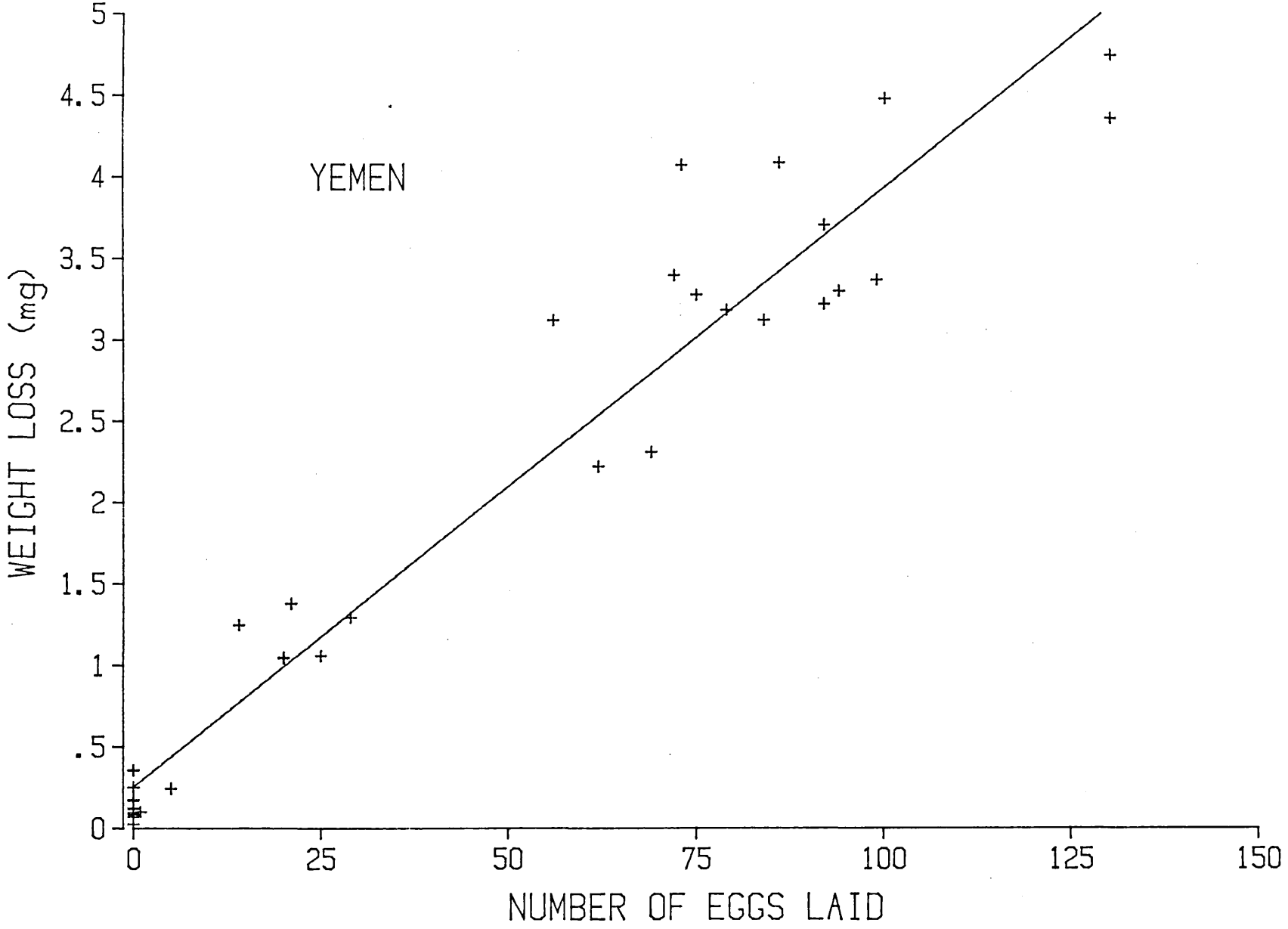

YEMEN
WEIGHT LOSS (mg)
NUMBER OF EGGS LAID
0
.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
25
50
75
100
125
150

Table 3.3: Regression equations for weight loss on egg laying.

Campinas:	Weight loss = 0.248 + 0.0219 x Eggs laid
IITA :	" " = 0.298 + 0.0215 x " "
Yemen :	" " = 0.252 + 0.0369 x " "

Weight loss is expressed in milligrams.
All regression coefficients are significantly different from zero ($p < 0.001$).

Table 3.4: Analysis of covariance for weight loss on egg laying.

Source of variation	DF	SS	MS	F	
Adjusted means					
Treatments	2	8.99	4.50	26.131	***
Error	150	25.81	0.17		
Total	152	34.80			
Homogeneity of regression coefficients					
Treatments	2	9.13	4.57	40.264	***
Error	147	16.67	0.11		
Total	149	25.81			

Table 3.5: Difference in weight loss between ovipositing and virgin females.

	Total number of eggs laid	Weight loss (mg)	n
VIRGIN	0.2 ± 0.2	0.802 ± 0.1	6
MATED	66.5 ± 9.4	2.339 ± 0.3	16

Values given are mean and standard error.

3.3.4. Emergence weight and fecundity

The relationship between emergence weight and fecundity is shown in Fig. 3.6. The regression equations are presented in Table 3.6. Results for the analysis of covariance are given in Table 3.7. The average weight at emergence of females of the three strains are given in Table 3.8.

3.3.5. 6-day egg laying

Fig. 3.7 shows the 6-day and lifetime egg laying totals. There is no significant difference between the 6-day total and the lifetime total.

3.3.6. Suppression of egg laying

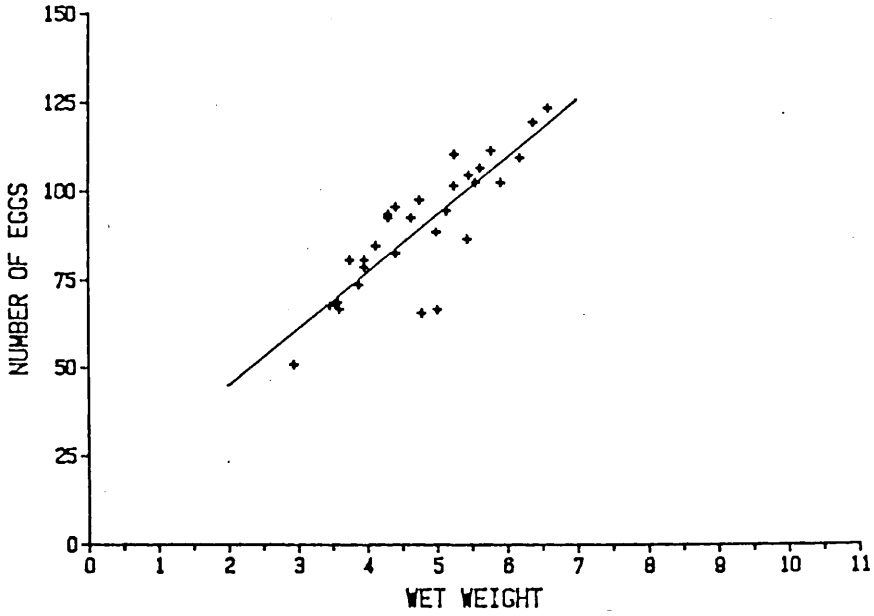
The result, in terms of egg laying, of restricting the number of oviposition sites is shown in Fig. 3.8. Table 3.9 shows where the differences between the control (egg laying by females on 40 cowpeas) and the treatment (egg laying by females on 2 cowpeas) were significant. Egg production for the Campinas and Yemen strains are shown in Fig. 3.9 and Table 3.10. Control and treatment values were not significantly different for the IITA strain at any time so only egg laying is shown (Fig. 3.8).

Figure 3.6: The relationship between emergence weight and egg laying.

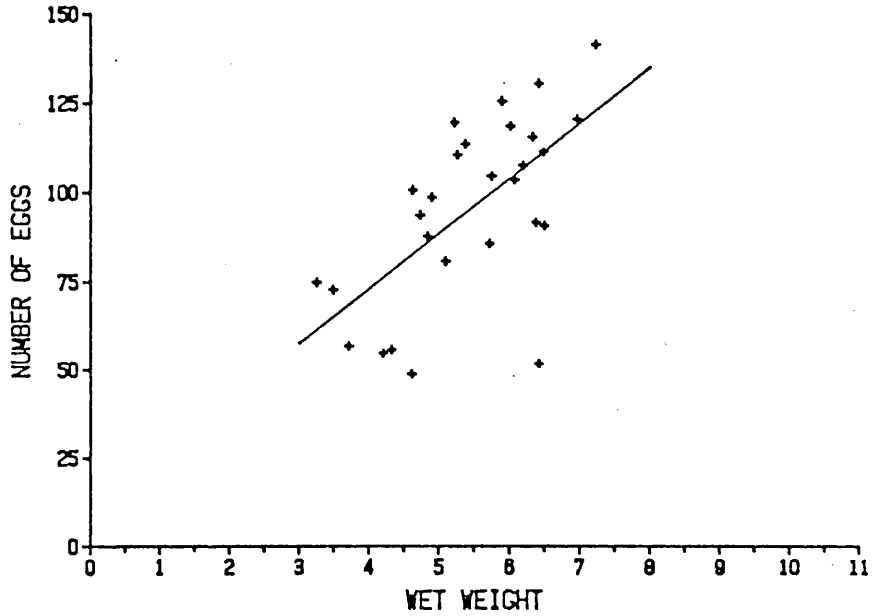
Numbers of eggs laid by individual females of known live weight at time of mating, shortly after emergence. Regression lines are plotted for each strain and all are significantly different from zero.

(a) Campinas
(b) IITA
(c) Yemen

(a)


NUMBER OF EGGS
WET WEIGHT
0
25
50
75
100
125
150
0 1 2 3 4 5 6 7 8 9 10 11

(b)


NUMBER OF EGGS
WET WEIGHT
0
25
50
75
100
125
150
0 1 2 3 4 5 6 7 8 9 10 11

(c)

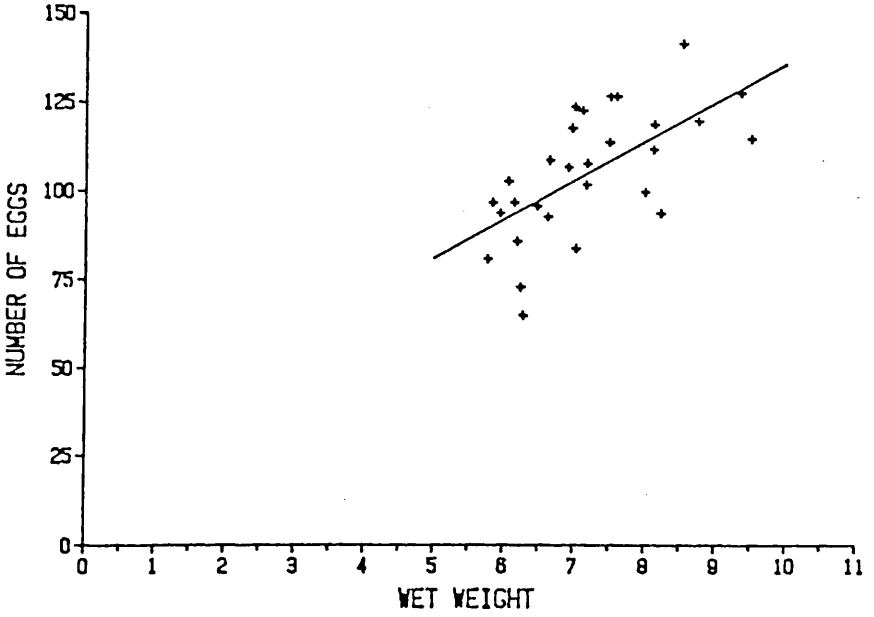

NUMBER OF EGGS
WET WEIGHT
0
25
50
75
100
125
150
0 1 2 3 4 5 6 7 8 9 10 11

Table 3.6: Regression equations for egg laying on emergence weight.

Campinas:	Egg laying	=	12.60	+	16.26	x	Emergence weight
IITA :	"	"	=	11.26	+	15.55	x	"	"
Yemen :	"	"	=	26.30	+	10.99	x	"	"

All regression coefficients are significantly different from zero ($p < 0.001$).

Table 3.7: Analysis of covariance for egg laying on emergence weight.

Source of variation	DF	SS	MS	F	
Adjusted means					
Treatments	2	2910.20	1455.10	6.436	**
Error	84	18990.76	226.08		
Total	86	21900.95			
Homogeneity of regression coefficients					
Treatments	2	472.66	236.33	1.034	N/S
Error	81	18518.09	228.62		
Total	83	18990.74			

Table 3.8: The weights at emergence of females of the three strains of C. maculatus.

Strain	Weight (mg)	S.Error	n
Campinas	4.788	0.171	30
IITA	5.428	0.199	28
Yemen	7.203	0.193	29

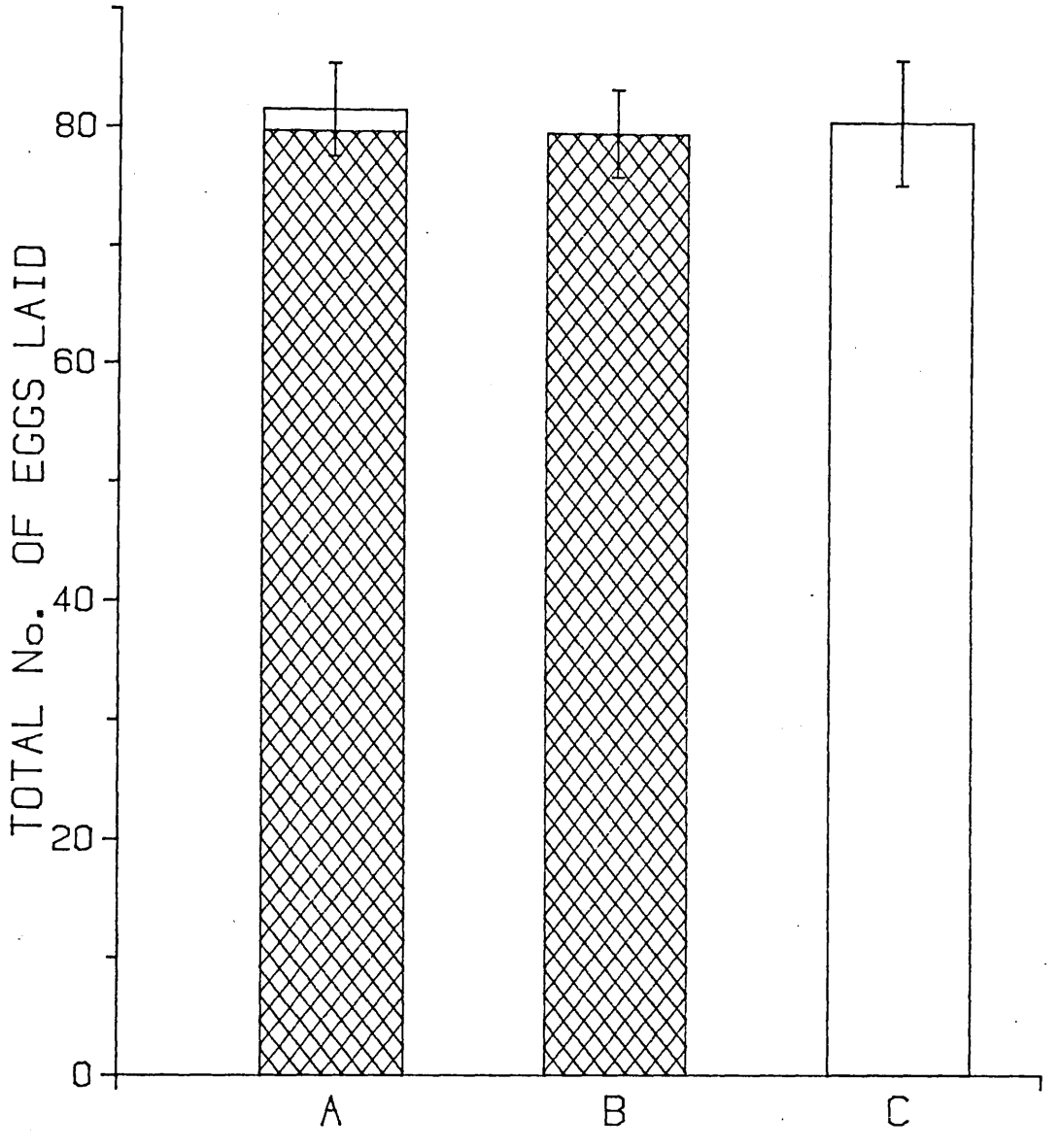


Figure 3.7: Average 6-day and lifetime egg laying totals.

Totals are mean values (± 1 S.E.) for Campinas females (20 replicates in each case) mated and placed on forty cowpeas.

A: Lifetime egg laying totals for females given fresh cowpeas after 6-days. Unshaded area represents the eggs laid after the sixth day

B: 6-day egg laying total.

C: Lifetime egg laying total for females left on 40 cowpeas, undisturbed until death.

There is no significant difference in the number of eggs laid by females over 6 days (B) and the number laid by females over their entire lifetime (C).

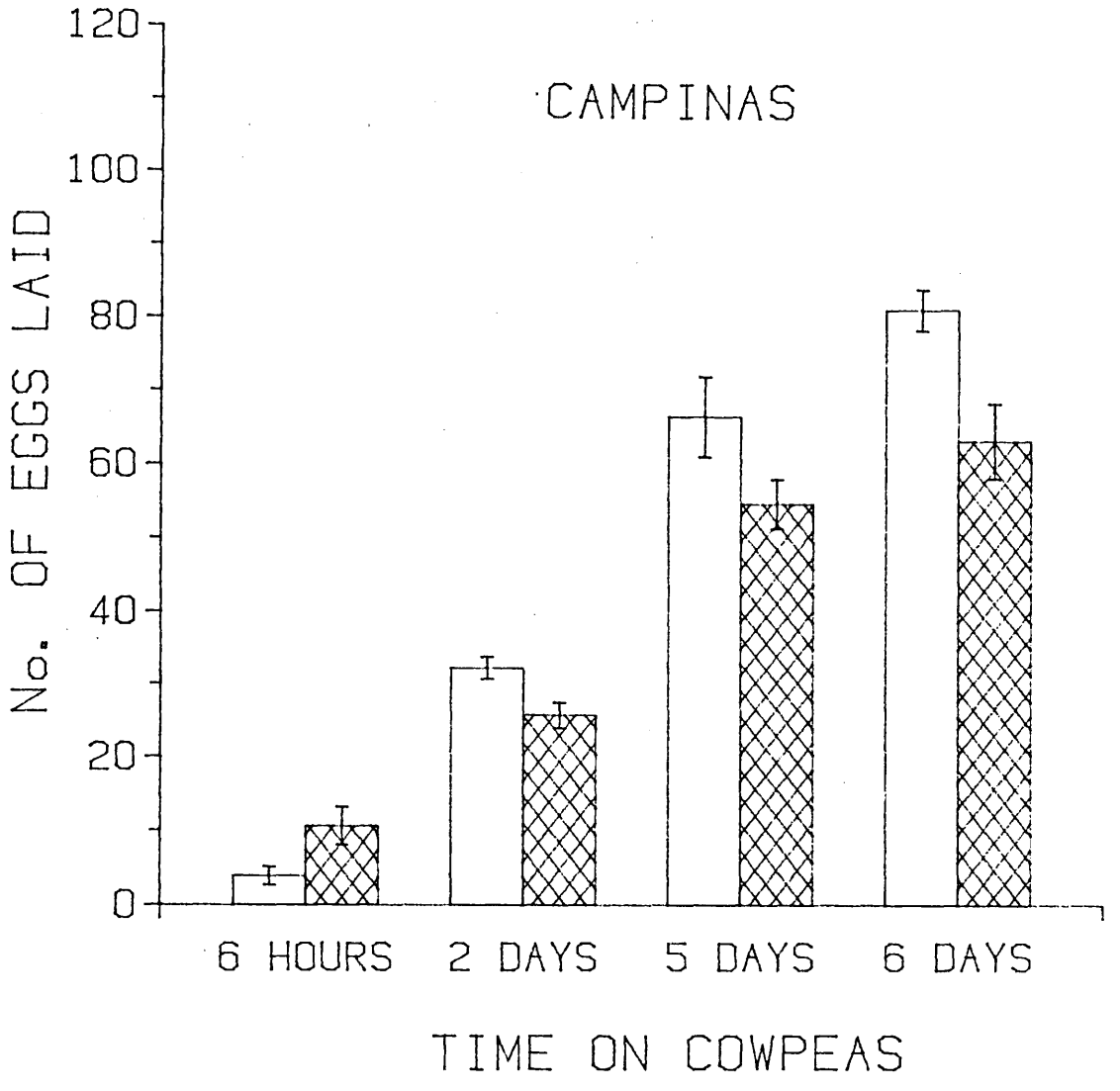


Figure 3.8: Effects on egg laying restricting the number of oviposition sites.

a) Campinas strain.

Females of each strain were mated and then placed on either 2 cowpeas (shaded bars) or 40 cowpeas (unshaded bars) for varying lengths of time. Total egg laying is a mean value from 10 replicates (± 1 S.E.).

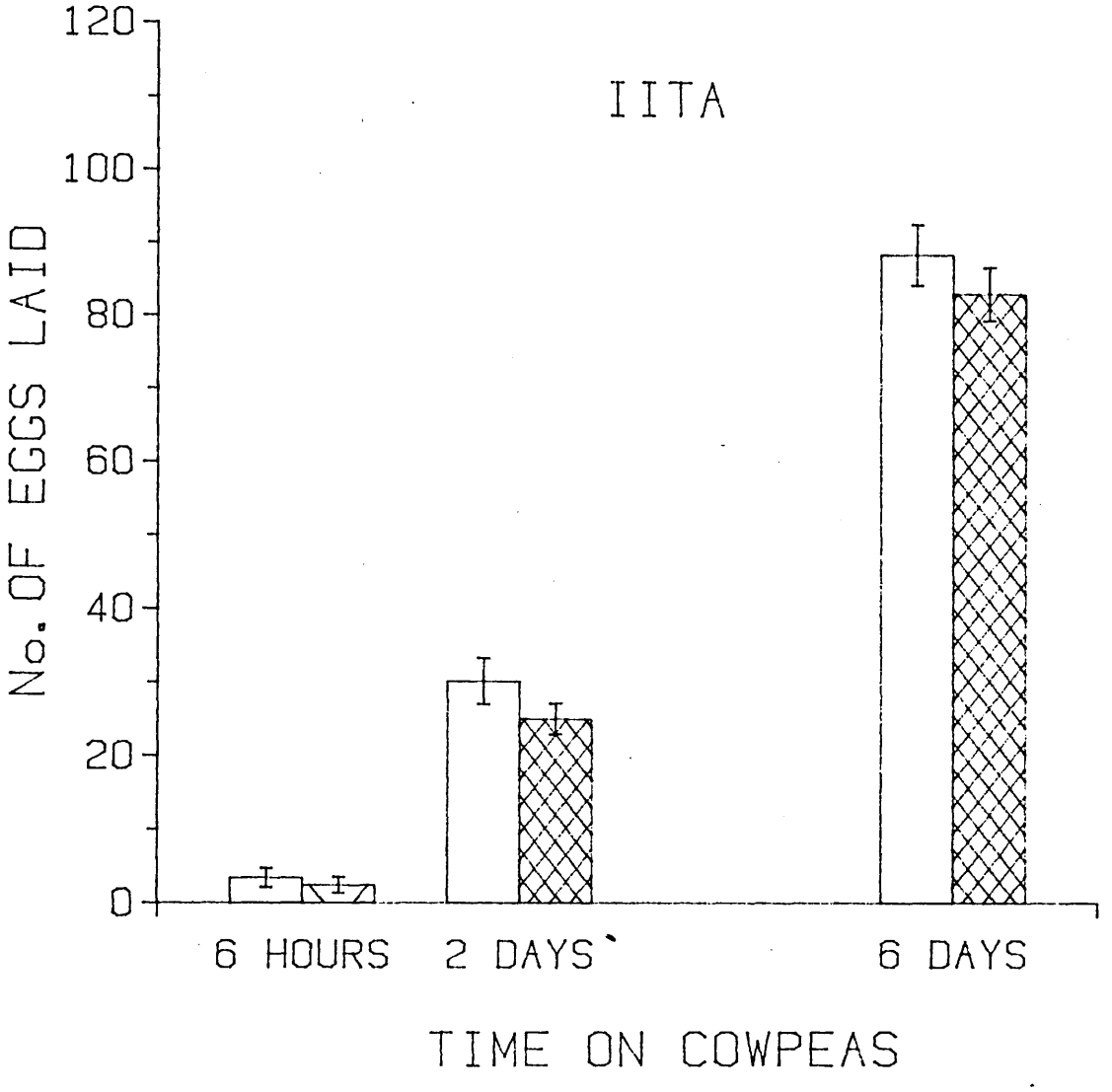


Figure 3.8: b) IITA strain

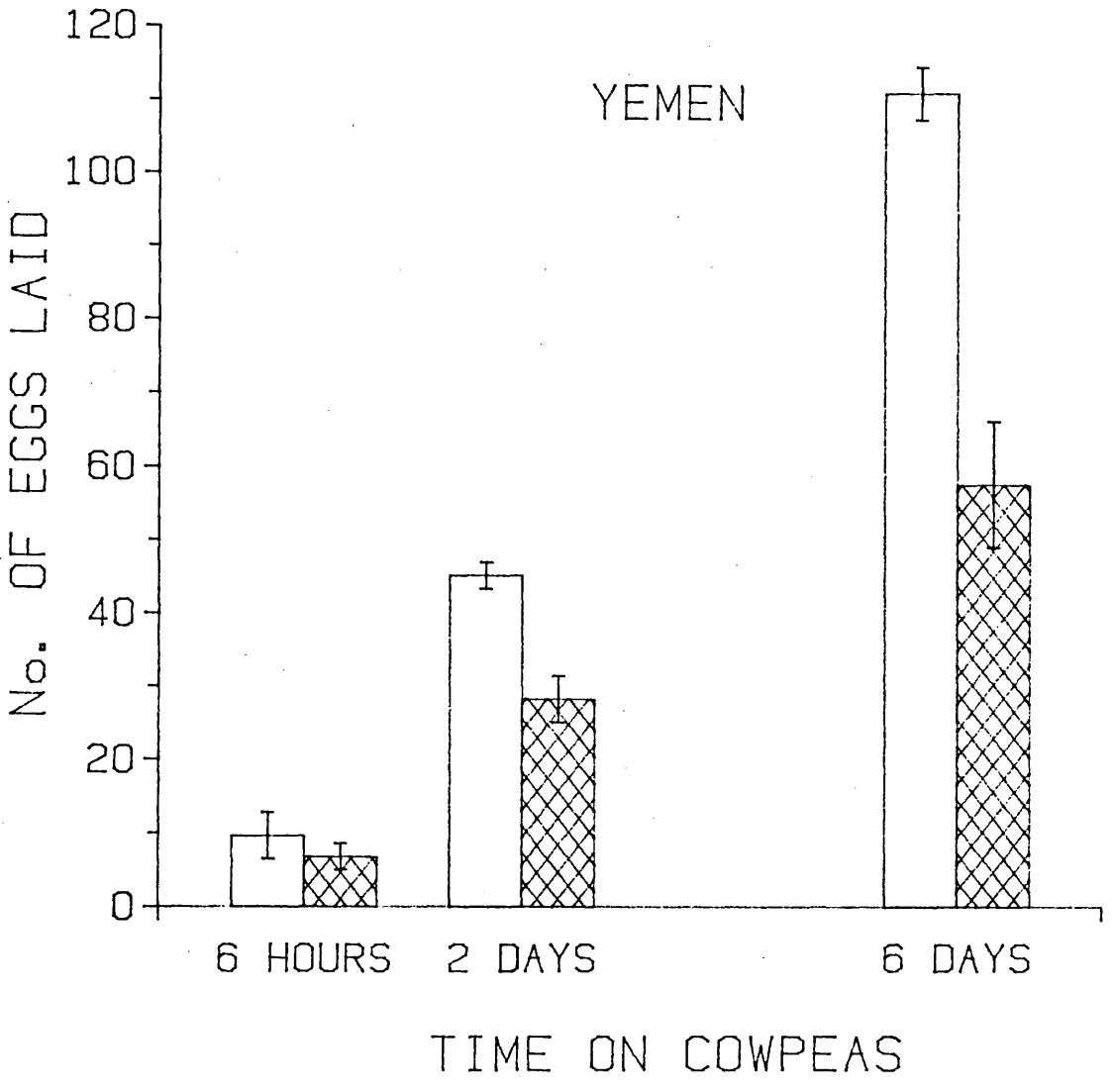


Figure 3.8: c) Yemen strain

Table 3.9: Significant differences between eggs laid by females on 2 and 40 cowpeas.

Time on cowpeas	6 hours	2 days	5 days	6 days
Campinas	*	*	*	**
IITA	N/S	N/S	--	N/S
Yemen	N/S	***	--	***

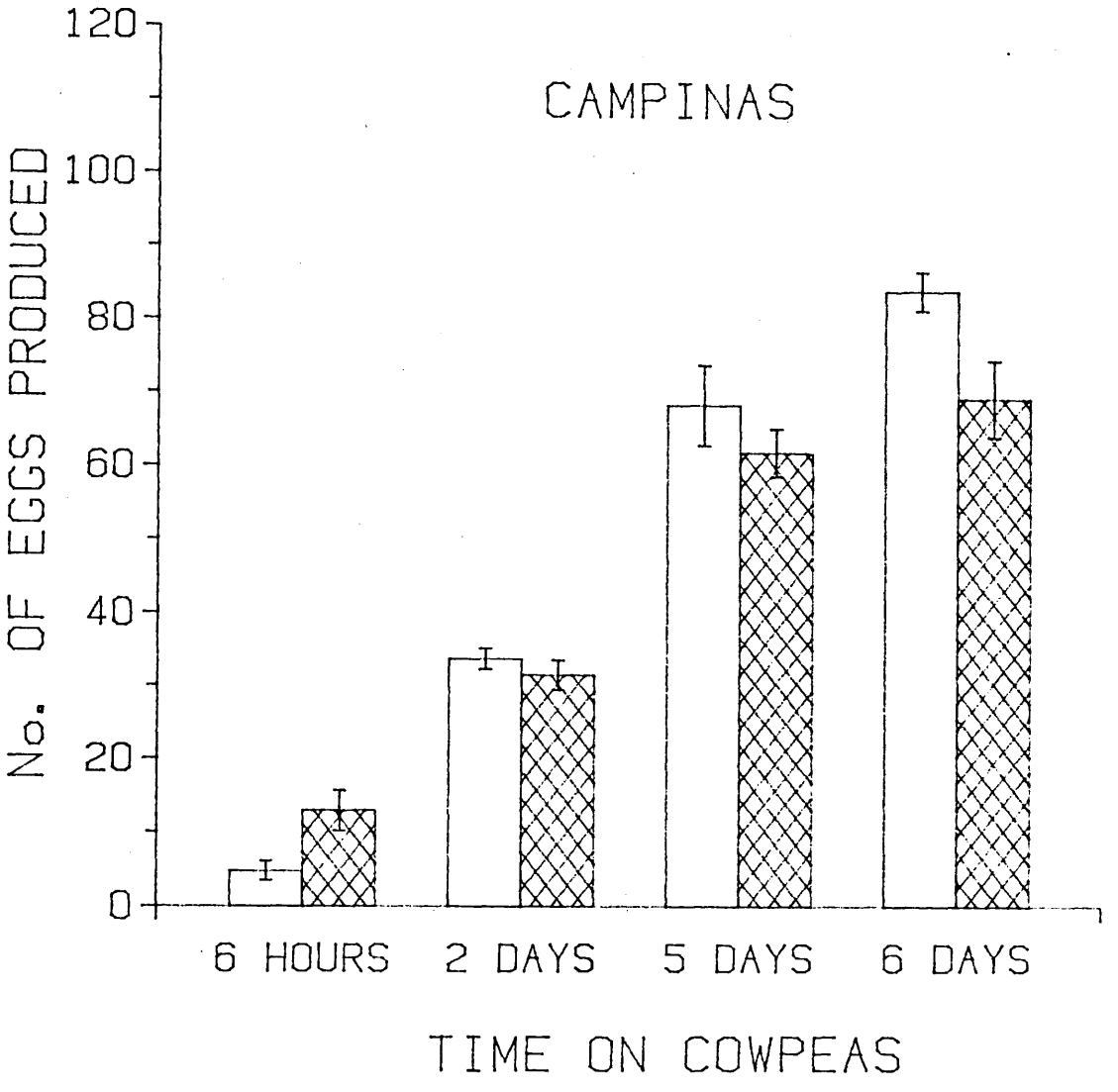


Figure 3.9: Effects on egg production of restricting the number of oviposition sites.

a) Campinas strain

Females of each strain were mated and then placed on either 2 cowpeas (shaded bars) or 40 cowpeas (unshaded bars) for varying lengths of time. Total egg production is a mean value from ten replicates (± 1 S.E.). Egg production is calculated as the number of eggs laid plus the number remaining in the lateral and median oviducts.

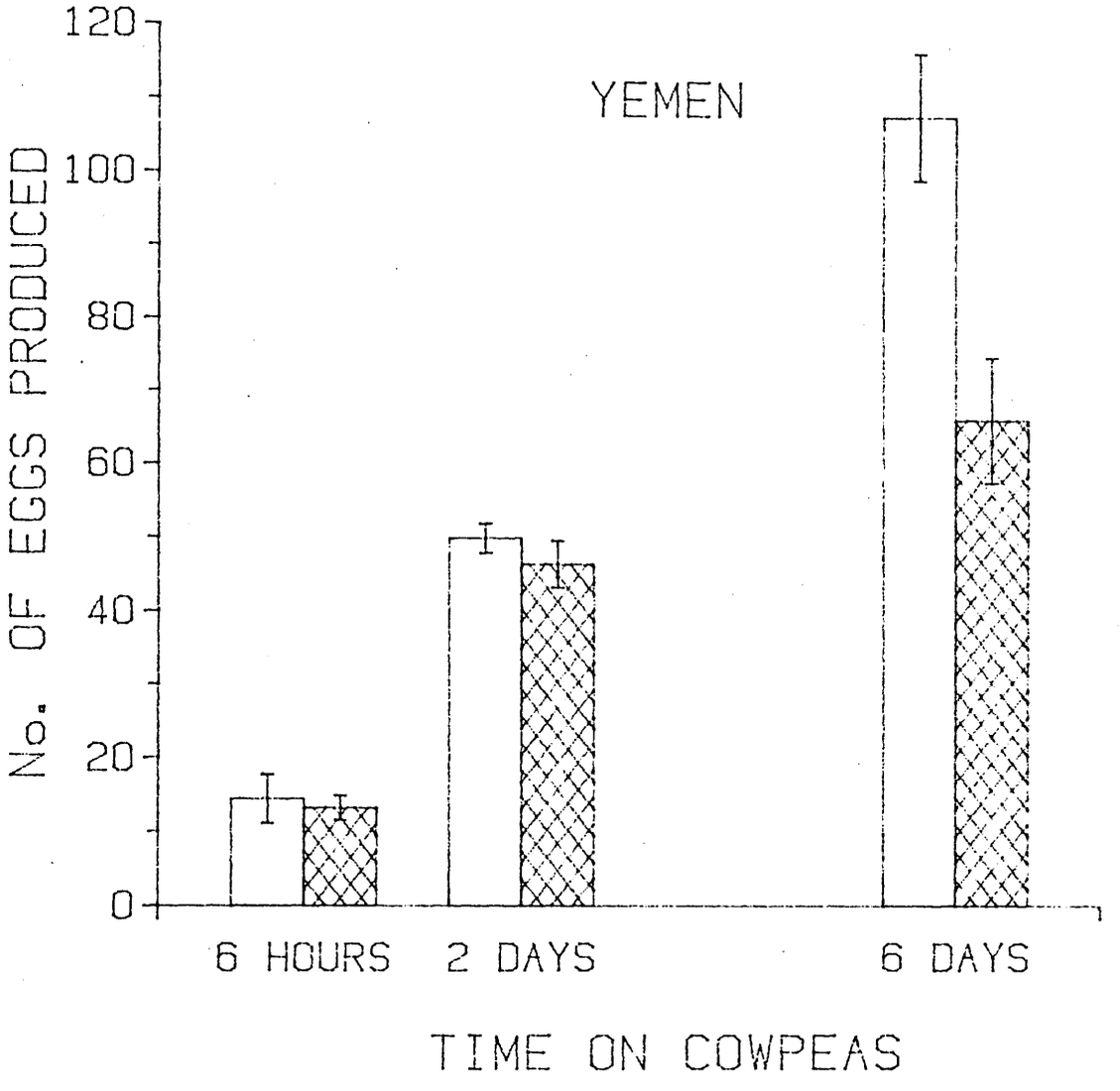


Figure 3.9: b) Yemen strain

Table 3.10: Significant differences between eggs produced by females on 2 and 40 cowpeas.

Time on cowpeas	6 hours	2 days	5 days	6 days
Campinas	*	N/S	N/S	*
IITA	N/S	N/S	--	N/S
Yemen	N/S	N/S	--	**

Table 3.11 shows the results of Student's t-test comparing the oocyte numbers of females on 2 cowpeas and on 40 cowpeas for each the three strains after 6 days.

Table 3.12 shows the results of the Mann-Whitney U-test comparing the percentage hatching of eggs laid by females on 2 and on 40 cowpeas over 6 days. The test was carried out for all three strains.

The effects of delaying the onset of oviposition are shown in Fig. 3.10. Preventing females from having access to cowpeas, whether before or after mating, significantly reduced their egg laying. Females mated before their 6 day isolation laid less eggs than those mated afterwards.

3.4. Discussion

In all strains, maximum egg laying occurred on the first day after emergence and then decreased daily. The first egg was laid sometimes within 2 hours of mating and with the exception of four cases out of 116, within 24 hours.

Larson and Fisher (1938) mentioned that oviposition frequently began within two hours and generally within the first 24 hours after emergence. They also stated that maximum oviposition occured on the first day and then decreased daily. This was further supported by Howe & Currie (1964), Utida (1972), Bellows (1982a) and Dick

Table 3.11: Comparison of oocyte numbers of females on 2 cowpeas and on 40 cowpeas after 6 days.

Strain	Student's t-test results		
Campinas	t = -0.617	df = 16	N/S
IITA	t = 0.000	df = 18	N/S
Yemen	t = -1.844	df = 17	N/S

Table 3.12: Comparison of hatching of eggs laid by females on 2 cowpeas and on 40 cowpeas after 6 days.

Strain	Mann-Whitney Two-Sample Test Results		
Campinas	U = 29.00	Z = 0.773	N/S
IITA	U = 34.50	Z = 0.898	N/S
Yemen	U = 19.00	Z = 1.696	N/S

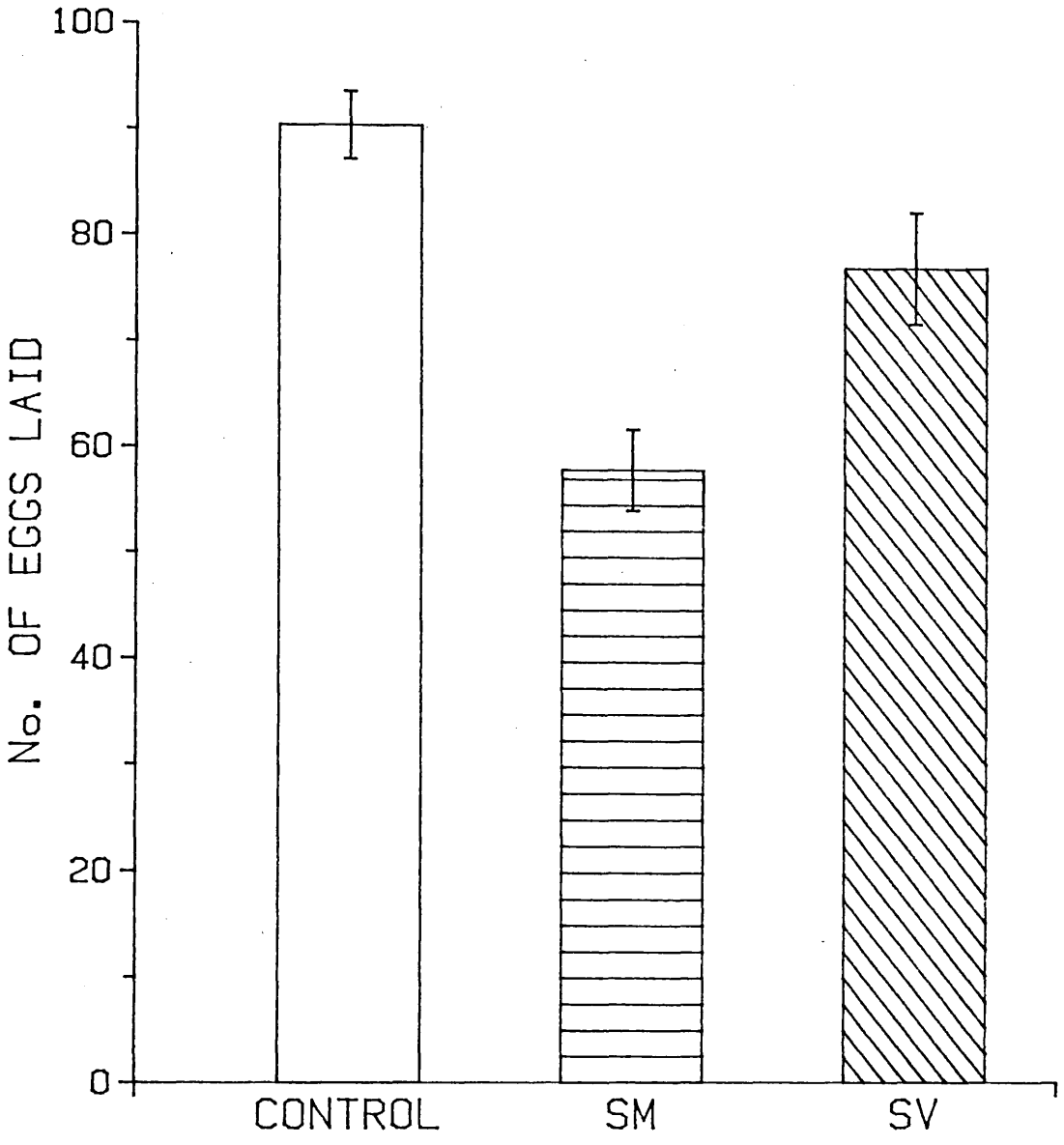


Figure 3.10: Result of delaying the onset of oviposition

Control — Mean number of eggs laid (± 1 S.E.) by Campinas females ($\bar{n}$ = 20) which were allowed to mate and begin oviposition immediately after emergence.

SM — Mean number of eggs laid (± 1 S.E.) by Campinas females ($\bar{n}$ = 20) which were allowed to mate immediately after emergence but which were prevented from ovipositing for 3 days.

SV — Mean number of eggs laid (± 1 S.E.) by Campinas females ($\bar{n}$ = 12) which were allowed to mate 3 days after emergence and then allowed to oviposit.

The totals for SM and SV are both significantly less than that of the control.

(1984). However Brauer (1945) described maximum oviposition as occurring on the second day after emergence. The results presented here, for the onset and daily pattern of oviposition, agree with those of most workers, for all three strains studied.

Under normal experimental conditions there was no significant difference between 6-day and lifetime egg laying. Thus, in experiments, removing females after 6 days allowed the reproductive system to be examined and sufficient time for egg laying but avoided excessive mortality among the beetles. In other experiments, where the reproductive system was not to be examined, lifetime egg laying was preferred as a measure of fecundity.

Lifetime egg laying totals were obtained from the experiment investigating the relationship between emergence weight and fecundity. These are given, along with the results of other workers, in Table 3.13 - this shows the variation in the reported values for the fecundity of C. maculatus and how values from the present study compare with them. Whilst care has been taken to select values from similar experiments these results are not necessarily comparable, the exact conditions are described in the particular publications and only major differences in experimental conditions are indicated.

Table 3.13: The fecundity of C. maculatus

No. of Eggs Laid		Remarks	Source
Average	Maximum		
91.2	124	Campinas	Present Study
95.6	142	IITA	" "
105.4	130	Yemen	" "
88.5	--	--	Larson & Fisher, 1938
94	--	On Peas	Brauer, 1945
---	80	--	El-Sawaf, 1956
About 60	--	--	Caswell, 1960
75.2	100	Range 35-100	Howe & Currie, 1964
---	90	Range 40-90	Booker, 1967
---	70	Range 60-70	Utida, 1972
84.6	--	On Mung Beans	Mitchell, 1975
40.5	--	--	Bellows, 1982a
72.2	--	Malawi Strain	Giga & Smith, 1983
73.1	--	Brazil Strain	Giga & Smith, 1983

Different fecundities have previously been described for different stocks of C. maculatus kept under the same conditions (Brauer, 1945). Throughout the course of the present study, where lifetime egg laying was recorded for all three strains, the Yemen strain consistently laid most eggs.

The higher fecundity of the Yemen strain was not due to a difference in the number of ovarioles between strains. Only four out of 79 Yemen females were found to have more than twelve ovarioles. Though it is interesting to note that only the Yemen strain showed any variation from the norm, this small variation could not cause the observed strain difference. The differences in fecundity must be due to other factors.

Ouedraogo & Huignard (1981) showed that in C. maculatus exposure to host-plant compounds and mating stimulated oogenesis and so increased the number of oocytes. This has also been described for A. obtectus (Huignard, 1979; Monge, 1983) and Z. subfasciatus (Pimbert & Pierre, 1983). After emergence, possibly due to the effects of mating and cowpeas, there was an increase in the number of oocytes per female over the first 24 hours (Fig. 3.4).

For the purposes of the experiment the instant of emergence of an adult was taken as the onset of adult life. However, this is not strictly correct since there may be a variable length of time between adult eclosion

from the pupal cuticle and emergence of the adult from the cowpea. During this time the reproductive system may develop to varying degrees and thus the reproductive systems of females may be at different stages of development on emergence from cowpeas. The effect of mating and cowpeas may be to reduce such differences by stimulating oocyte development in females with less developed reproductive systems.

A linear trend could be identified after the first 24 hours because the initial differences between females became less important. The average number of oocytes decreased with age after the first day.

Sidhu, Kaurs & Kumar, (1980) described weight loss with time in C. maculatus females. Utida & Takahashi (1958) also described a decrease in body weight of adults of C. maculatus with time and mentioned that water content was almost constant throughout life. Wightman (1978) stated that water loss in Callosobruchus analis was low because water lost by transpiration was replaced by metabolic water; this is likely to be the case in C. maculatus. Under storage conditions adults would not feed (Dobie, 1981) and they had no opportunity to do so during the experiments described here. Only metabolic activity, transpiration, defaecation (of material present in the gut at emergence - pers. obs.) and oviposition are likely to make large contributions to weight change.

Virgin females which rarely lay eggs, and even if they do so very few, infertile eggs late in their lives (Ouedraogo & Huignard, 1981; Credland, pers. comm. and pers. obs.), lose much less weight than do ovipositing females. This is shown in Table 3.5. Since the only differences between virgin and ovipositing females are the latter's reproductive activities then the difference in weight loss must stem from them. Oviposition thus accounts for a major portion of the weight loss of females (through whatever mechanism).

There was no significant difference between the regression coefficients for weight loss on egg laying of the Campinas and IITA strains but these two were different from the Yemen strain. A Student-Newman-Keuls multiple comparison test among the adjusted mean numbers of eggs laid (to allow comparison) indicated that the Yemen strain again differed significantly from the other two. Yemen females tended to lose more weight when laying similar numbers of eggs than Campinas or IITA females and as the numbers of eggs laid increased so Yemen weight loss increased at a greater rate.

The reason for this difference is not obvious. Yemen females are generally heavier than those of the other two strains (Table 3.8) and they may expend more energy than the other two strains on the maintenance of bodily functions other than egg laying. Alternatively there may be differences in the weights of eggs, with

Yemen females laying heavier eggs, but this would be difficult to test.

A major portion of weight loss is due to the expenditure of energy reserves on oviposition. Utida & Takahashi (1958), Caswell, (1960), Sidhu et al (1980), Sharma, Jit & Shasma, (1983), and Puri & Sharma (1984) have all described the decrease of one form of energy reserve or another with age in C. maculatus. Wightman (1978), working on Callosobruchus analis (which has comparable reproductive habits to C. maculatus) stated that eggs account for 10% of the expenditure of initial energy content of the beetle and metabolic processes, which presumably include reproductive processes, a further 55%; the rest of the initial energy content remained in the cadavers.

It was noted during the course of the present experiment that in older C. maculatus females the reproductive system had a withered and effete appearance. Fat body, which was very prominent in newly emerged females, had almost disappeared in females of six and seven days old. Unless they feed, females have no way of replacing food reserves and so energy available for egg laying decreases.

As energy reserves are depleted either the number of eggs being produced by the ovaries must decrease, egg production must eventually stop or the eggs must get smaller. The results show a reduction in the number of

oocytes in the ovaries with time (Fig. 3.4). Ahmed, Elbadry & Salem, (1976b) describe a reduction in length of mature oocytes of C. maculatus with age and there is some evidence that the volume of mature oocytes decreased (pers. obs.). Bhaskar, Koul & Tikku, (1976) have obtained similar results for C. chinensis.

These experimental results confirm and expand upon previous observations of the degeneration with age of the reproductive systems of non-feeding females of C. maculatus. The reduction of energy reserves and the reduction in the number of oocytes appear to be responsible for the daily egg laying pattern of this species under storage conditions.

A positive relationship between adult weight and fecundity has been demonstrated in other insect species (Snyman, 1949; Ullyett, 1950). It was found that C. maculatus demonstrates a strong positive relationship between the weight at emergence and fecundity (Fig. 3.6) in all three strains.

There was no significant difference between the regression coefficients of the three strains but a Student-Newman-Keuls multiple comparison test, with an adjusted mean fecundity, indicated that the Yemen strain was significantly different from the other two strains. This indicates that at comparable weights Yemen females lay significantly fewer eggs than either Campinas or IITA females. In practice, Yemen females are generally

heavier than those of the other strains (Table 3.8) and have the higher fecundity. Females of Pieris rapae having the same weight but different genotypes have also recently been shown to have different fecundities (Gilbert, 1984).

Because the C. maculatus females in this study were not fed it is not surprising that adult weight has such a strong relationship with egg laying.

Dick & Credland (1984) described the suppression of egg laying by females of the Campinas and Yemen strains on a small number of cowpeas. They also stated that females of the IITA strain did not suppress egg laying under similar conditions. The present study confirmed these observations (Fig. 3.8) and was extended to investigate the effect of such suppression on egg production, changes in oocyte numbers and egg hatching.

After 6 hours, Campinas but not IITA or Yemen females on 2 cowpeas laid and produced significantly more eggs than females on 40 cowpeas. It is not known why this happened or why this was not found in the other two strains.

Study of egg production in Campinas and Yemen females indicated something of the mechanism of suppression observed at two days and after. Although egg laying was significantly reduced after 2 days, egg production was not. This was because eggs were stored in

the lateral oviducts. After 6 days, even though some eggs were stored in the lateral oviducts, egg production was less in females on 2 cowpeas than in females on 40. In this case either egg production was depressed or oocytes or eggs were resorbed.

At first, when a female was presented with a limited number of oviposition sites it apparently avoided 'overcrowding' these by retaining some eggs in its lateral oviducts. Although under other circumstances a female may sooner or later attempt to find fresh oviposition sites in this case, as the egg load on the cowpea built up, egg production was decreased by some means.

The method by which this reduction is brought about is not known. There is no significant difference between the oocyte number of females on 2 and 40 cowpeas in either Yemen or Campinas so the decrease in egg production was apparently not achieved by reducing the number of maturing oocytes; it may be that the development of oocytes is suspended. An oostatic hormone has been demonstrated in Musca domestica which can arrest the development of certain stages of oocytes when mature eggs are retained (Adams, Hintz & Pomonis, 1968). A similar mechanism could operate in C. maculatus.

Mann-Whitney two-sample tests show no difference between the percentages of eggs hatched in the control and treatment after 6 days. Even though eggs may hatch with equal frequency post-embryonic mortality is higher in cowpeas with large numbers of hatched eggs (Dick & Credland, 1984).

In the field, situations can occur which leave females with little choice but to deposit a large number of eggs on a few cowpeas. For instance, small numbers of cowpeas are frequently left in storage areas after they have been emptied. Whilst it is better, in terms of reproductive fitness, for a female to disperse as many eggs as possible on a large number of cowpeas,it may be more effective for a female to limit egg laying when it is on a small number of cowpeas.

Credland et al (1986) found that as the number of eggs laid on a cowpea by one female increased so the total egg laying by the F_1 sibling females emerging later, from the same cowpea, also increased. Such egg laying by the offspring did not, however, increase indefinitely. Above a certain larval density no further increase in the F_1 egg laying occurred and there may have been a decrease (although this was unclear). Thus, in terms of the total number of eggs laid by its female offspring the female parent may be more successful if its own egg laying is suppressed once a certain egg density has been reached.

The argument presented by Credland et al (1986) only applied to the Campinas and Yemen strains. Where females of the IITA strain were concerned, in the same circumstances (up to a hatched egg density of 22 per cowpea), the theoretical total egg laying by F_1 sibling females from a single cowpea did not decrease or level off. For the IITA strain there was no theoretical optimum number of eggs per cowpea which produced the maximum number of eggs from emerging F_1 females.

The suppression of egg laying by a female (of the Campinas or Yemen strains) restricted to a small number of cowpeas would only improve the reproductive fitness of a female if all the eggs already laid on the cowpeas were its own. If the eggs which a female encountered were those of other females then laying its own eggs on the cowpeas, however unlikely they were to survive, could only improve its reproductive fitness (which would otherwise be zero with respect to these cowpeas). If females decide whether or not to oviposit on cowpeas already laden with the theoretical 'optimum' number of eggs then they must be able to distinguish between their own eggs and those of others. There are no references to this subject for C. maculatus but Avidov, Applebaum & Berlinger, (1965a) stated that females of C. chinensis do not distinguish between their own and other eggs. If a C. maculatus female cannot distinguish between its own and other eggs then it may respond to all eggs in a similar way and avoid exceeding the 'optimum' number of

eggs per cowpea.

The results partly explain why both Campinas and Yemen may suppress egg laying on a limited number of cowpeas and why IITA does not. The explanation is imperfect however. Campinas females actually laid an average of 31.8 eggs/cowpea on two cowpeas after 6 days (28.7 hatched eggs/cowpea) and Yemen females 28.9 eggs/cowpea (26.8 hatched eggs/cowpea) (Fig. 3.8). Both of these egg densities were beyond the theoretical optima which will enable maximum egg laying by F_1 sibling females emerging from the same cowpea. It may be that the production of male adults plays an important part in the strategy of a female; the increased fecundity of males (expressed by the number of eggs that they fertilise) may require slightly different developmental conditions. Males were not considered in the explanation by Credland et al (1986) of the suppression of egg laying on a reduced number of cowpeas.

Suppression of egg laying by delaying the onset of oviposition has been observed in several species (Avidov et al, 1965a; Huignard, 1970; Bell, 1971; Bell & Bohm, 1975; Tyndale-Biscoe & Watson, 1977; Biemont, 1979; Allemand, 1983). Delaying the onset of oviposition for 6 days (a time which would have allowed a normal number of eggs to be laid in ideal circumstances) for both mated and virgin Campinas females significantly reduced the number of eggs laid.

The reduction of egg laying is less in females prevented from mating than in those mated. Mated females may expend more energy in searching for cowpeas than virgins; virgin females tend to be less active in the tubes (pers. obs.). In A. obtectus material provided by the male, in the spermatophore, is taken into the haemolymph (Huignard, 1983) and incorporated into developing oocytes. Thus, in A. obtectus the male contributes to egg production with its spermatophore. In C. maculatus similar material may be depleted in a mated female prevented from egg laying whereas the virgin females (mated with newly emerged males) would have a source of fresh spermatophore material.

The mechanism of suppression by delaying the onset of oviposition or by restricting the number of oviposition sites available to a female is rarely discussed. Oosorption has been identified in some cases (Bell, 1971; Bell & Bohm, 1975; Tyndale-Biscoe & Watson, 1977) but there are no references to this in C. maculatus or other bruchids.

Retention of eggs in the lateral oviducts by C. maculatus may reduce egg production and this may be irreversible. Alternatively, energy may be expended in searching behaviour and supporting bodily functions thereby depriving the female of resources which would otherwise be expended on egg production. Either of these could be reasons for the reduced egg laying of females.

Although in some circumstances females would probably leave their immediate environment to search for new hosts these experiments do resemble situations which can occur such as a female being trapped in a store. The changes in egg laying behaviour described here represent the attempts of females to achieve the maximum level of fecundity possible in less than perfect conditions.

Chapter 4

ARTIFICIAL OVIPOSITION SUBSTRATES, HOST-PLANT EXTRACTS AND DIFFERENT SUBSTRATE ARRANGEMENTS.

4.1. Introduction

In experiments investigating the fecundity and choice of oviposition site of Callosobruchus maculatus it is necessary to present females with cowpeas of similar size and shape so that other variables may be altered and the results interpreted correctly. However, despite attempts to standardise cowpeas for experiments there are always slight differences, such as roughness, which cannot easily be eliminated. Whilst these differences are presumed to be unimportant for most experiments it is still occasionally desirable to have an oviposition substrate which is even more uniform than standardised cowpeas.

In these cases it was proposed that cowpeas should be replaced with glass beads as these vary little in size or texture and can easily be cleaned before and after use. Before glass beads could be substituted for cowpeas, though, it was necessary to demonstrate that they provided a surface for oviposition of comparable attractiveness to cowpeas.

In such an experiment the effect of arranging cowpeas in a single layer in a Petri dish could also be investigated. It was thought that females may expend a lot of energy moving among the cowpeas stacked in tubes. The hypothesis was that females would be able to move more freely among cowpeas in a single layer, in this way they would save energy which might be used in egg production.

Monge (1983) obtained a seed-coat extract from Phaseolus vulgaris which stimulated oviposition by Acanthoscelides obtectus. An experiment was therefore designed to see whether a more sophisticated artificial substrate than plain glass beads could be prepared by coating glass beads with a similar extract taken from whole cowpeas.

Since Monge (1983) had obtained extract from seed-coats only, it seemed likely that an effective cowpea extract might be obtained by soaking cowpeas for shorter lengths of time than the original period, 24 hours, of the first cowpea extract experiment. The effective components of the extract might be found in the surface of the cowpea and be quickly washed into solution, whereas prolonged soaking may have removed material from below the testa which was not normally encountered by adult beetles.

This experiment, in which the extraction times were varied, gave unexpected results. Egg laying totals for the females on beads coated with a 6 hour or a 24 hour extract were lower than expected whilst those of females on beads coated with a 10 minute extract were as high as egg laying totals of females on cowpeas. This suggested that there may be some deterrent to oviposition caused by the physical nature of the extracts or by some chemical substance extracted in sufficient quantity after 6 hours. Host-plant substances which inhibit oviposition are of particular interest because of their possible value in pest control (Bodde, 1982).

By varying the concentrations of extracts collected over 10 minutes and 24 hours more information might be provided on the value of these extracts as oviposition deterrents. If the effect was physical then increasing the concentration of the 10 minute extract might cause inhibition of oviposition and lowering the concentration of the 24 hour extract might remove such inhibition. Increasing the concentration of the 10 minute extract might also concentrate a chemical oviposition deterrent to the extent that it became effective. However, if the suppression of oviposition was caused solely by a chemical extracted only after 6 hours, then increasing the concentration of the 10 minute extract would have no effect.

4.2. Materials and Methods

4.2.1. Materials

Since the beetles were to be left in the Petri dishes for 6 days the dishes were lined with emery cloth to prevent oviposition on the plastic. Glass beads were obtained from BDH Chemicals Ltd., Poole, England and were between 6.5 and 7.5 mm in diameter. These size beads were used since they were about the same size as cowpeas. Analar acetone (BDH Chemicals Ltd., Poole, England) was used throughout the experiments. Acetone, rather than another solvent, was used because Monge (1983) found this to elicit the greatest response in experiments with Acanthoscelides obtectus.

In the rest of the experiments described in this thesis, unless otherwise stated, only beetles of the Campinas strain were used. This was to prevent needless repetition of experiments. Where strain differences were thought likely to be important then all three strains were used.

4.2.2. Methods

Glass beads as an oviposition substrate and different arrangements of oviposition substrates

10 Petri dishes were prepared, each containing 40 clean glass beads arranged randomly in a single layer. 10 glass tubes were prepared also containing 40 clean glass beads. This was repeated with cowpeas; 40 cowpeas

in each of 10 Petri dishes and 40 cowpeas in each of 10 glass tubes.

A newly emerged and mated Campinas pair was placed in each container. The Petri dishes and glass tubes were then placed in the CTH room and left, undisturbed, for 6 days. Afterwards the beetles were removed and the eggs counted. Those on the cowpeas were left for 7 days to allow them to hatch.

Effect of a cowpea extract on oviposition

500 cowpeas with whole, unbroken seed coats were placed in a conical flask and covered with 250 ml of acetone. The flask was stoppered and left standing for 24 hours without being shaken. After 24 hours the liquid was decanted; this was the cowpea extract.

The cowpea extract was placed in a 500 ml evaporating flask with 400 glass beads and the acetone evaporated off at 50 °C under reduced pressure. Some liquid was left after this process, which may have been water present in the original acetone or substances with higher boiling points which had been extracted from the cowpeas. In any case this liquid was poured off. The beads were then dried at room temperature in an airflow and occasionally turned to ensure even coating with extract. Some of the extracted material must have been poured off with the unevaporated liquid but as the purpose of the experiment was merely to demonstrate the

presence of a substance or substances which stimulated oviposition this was not important.

Another 400 glass beads were prepared as a control using 250 ml of acetone only. This was evaporated off in the rotary evaporator at 50 °C; all the liquid was evaporated off.

40 glass beads, all coated with extract, were placed in each of 10 glass tubes; another 10 glass tubes were prepared in the same way using the control glass beads. 10 more tubes were prepared with 40 cowpeas in each and another 10 each with 40 of the cowpeas from which the cowpea extract was made (the 'extracted cowpeas').

A newly emerged and mated Campinas pair was placed in each of the forty tubes and these were left, undisturbed, for 6 days in the CTH room. The beetles were then removed and the eggs counted a week later.

Time required to obtain an effective cowpea extract

Four lots of 500 cowpeas, each with whole, unbroken seed coats were placed in separate conical flasks and each covered with 250 ml of acetone. The flasks were allowed to stand for differing lengths of time. The cowpeas in one flask were left undisturbed for 24 hours and in the other three for 6 hours, one hour or ten minutes.

At the end of the allotted time period the liquid was decanted and the cowpeas were rinsed with 50 ml of fresh acetone which was then added to the original liquid. These four lots of liquid were the different cowpea extracts.

The cowpea extracts were placed, separately, into a rotary evaporator each with 400 clean glass beads and the acetone evaporated off at 35 °C. Another 400 glass beads were prepared as a control using acetone only. The glass beads were prepared in sequence beginning with the control followed by the ten minute extract, 1 hour extract, 6 hour extract and, finally, the 24 hour extract (with the evaporating flask being cleaned each time). The acetone evaporated quickly and no liquid was left.

The glass beads were placed in sealed containers in a refrigerator (at 4 °C) until they were required. Forty beads coated with the same extract were placed in each of ten glass tubes (lined with emery cloth in the usual way). Forty control glass beads were also placed in each of ten glass tubes. In addition 40 fresh cowpeas (sieved and conditioned) were placed in each of ten glass tubes.

Gloves were worn when handling the beads to prevent contamination and a different pair was used for beads coated with different extracts. These precautions were taken for all subsequent experiments in which glass beads or cowpeas were coated with cowpea extracts or other substances.

The glass tubes containing cowpeas and those containing glass beads were placed in the CTH room to allow their temperature to stabilise.

Two days later, 60 mated pairs of Campinas were collected in the usual way and placed in the tubes. The beetles were left, undisturbed, on the cowpeas or glass beads for 6 days after which they were killed and frozen.

The eggs laid were counted and each female was dissected to find the number of eggs in the lateral and median oviducts. This gave the total number of eggs produced after six days.

Different concentrations of cowpea extract

10 minute extracts

In the previous experiment the ten minute extract was made by placing 500 cowpeas (approximately 130 g) with whole, unbroken seedcoats in 250 ml of acetone for 10 minutes and then rinsing with 50 ml of fresh acetone to give 300 ml of extract.

In this experiment four different concentrations of 10 minute extract were made. An amount of extract fifteen times that of the original amount was prepared using 1950 g of cowpeas soaked for 10 minutes in 3750 ml of acetone and then washed in 750 ml of acetone to give 4500 ml of 10 minute extract.

Of this extract 300 ml were evaporated onto 400 clean glass beads to give 10 minute extract at the original concentration on the beads. Following this 600 ml, 1200 ml and 2400 ml of the extract were evaporated onto separate lots of 400 glass beads to give extract on the beads at 2X, 4X and 8X 'normal' 10 minute concentration. In the event the 8X 'normal' concentration was prepared separately due to practical difficulties involving the large amounts of cowpeas and acetone.

The extract was refrigerated (at 4 °C) for a short while prior to its being evaporated onto the glass beads. Following their preparation, the four lots of glass beads were also stored in a refrigerator (each lot separately, in sealed plastic containers) until they were required.

In addition to the glass beads with extract, 400 glass beads were prepared as a control using 300 ml of fresh acetone.

24 hour extracts

In the previous experiment the 24 hour extract was prepared by placing 500 cowpeas (approximately 130 g) in 250 ml of acetone for 24 hours and then rinsing them with 50 ml of fresh acetone to give 300 ml of extract.

In this experiment four different concentrations of 24 hour extract were prepared. 260 g of cowpeas, with whole, unbroken seedcoats, were soaked for 24 hours in 500 ml of acetone and then rinsed with 100 ml of fresh acetone to give 600 ml of 24 hour extract.

Of this 600 ml, 300 ml was evaporated onto 400 clean glass beads to give 24 hour extract at the original concentration on the beads. 150 ml of the remaining extract was diluted with 150 ml of fresh acetone and evaporated onto 400 glass beads to give 24 hour extract at half the normal concentration. A similar procedure was carried out to give two more lots of glass beads at 1/4 and 1/10 of the 'normal' 24 hour concentration. Another 400 glass beads were also prepared as a control using 300 ml of fresh acetone.

The glass beads were then transferred to glass tubes. In each tube were placed forty of the appropriate glass beads to give five lots of ten tubes each containing beads of a given concentration of extract or control. In addition 10 tubes were prepared each containing 40 sieved and conditioned cowpeas.

The experiment itself was conducted in two parts. First a mated Campinas pair was placed in each of the sixty tubes containing glass beads coated with the different 10 minute extracts (including control glass beads and cowpeas) and secondly a pair was placed in each of the sixty '24 hour' tubes. In each case the pairs

were left in the tubes, undisturbed, for 6 days before being removed ,killed and frozen. The number of eggs laid by each female was counted; the dead females were dissected and the numbers of eggs stored within the oviducts counted to give total egg production.

4.3. Results

4.3.1. Use of glass beads and different substrate arrangements

Fig. 4.1 shows the mean egg laying of females on glass beads and cowpeas in either Petri dishes or tubes. Table 4.1 gives the analysis of variance for these results.

The distribution (as measured by the ratio of mean crowding to the mean) of eggs laid by females on cowpeas in tubes or on cowpeas in Petri dishes was compared using the Mann-Whitney U-test. There was no significant difference between the two distributions of eggs.

4.3.2. Demonstration of a host-plant effect

Fig. 4.2 shows the mean egg laying of females subjected to the different treatments described in the method. Table 4.2 gives the analysis of variance of these results.

The distribution (as measured by the ratio of mean crowding to the mean) of eggs laid by females on glass beads coated with cowpea extract was compared with that

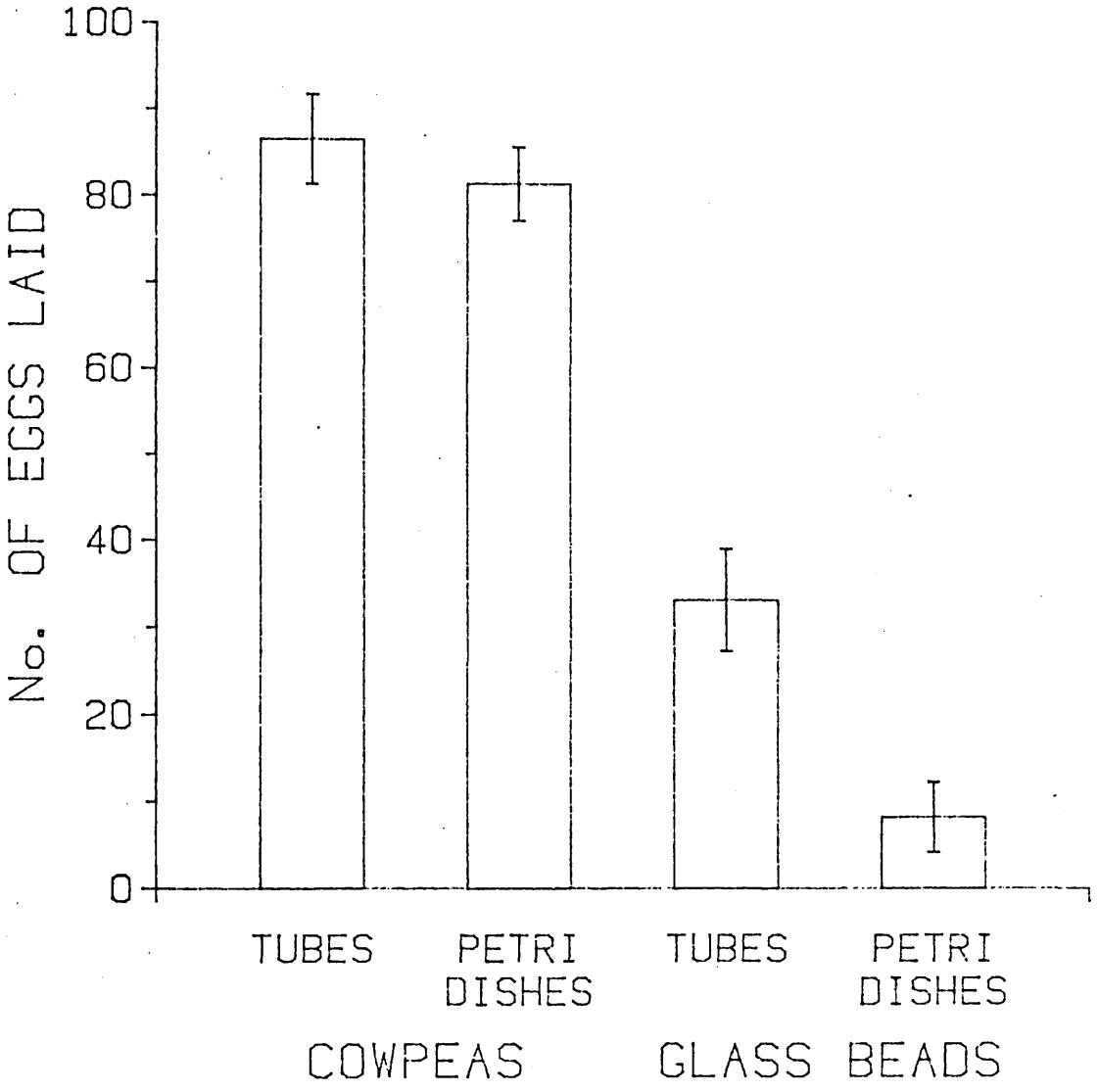


Figure 4.1: Egg laying on glass beads or cowpeas in either tubes or Petri dishes.

Campinas females were mated and then placed on either forty glass beads or forty cowpeas in a tube or in a Petri dish. Total egg laying is a mean value from 10 replicates (± 1 S.E.) in which females were allowed to oviposit for 6 days.

Table 4.1: Analysis of variance for egg laying on different substrate types and arrangements.

Source of variation	DF	SS	MS	F	
Treatments	3	43445.600	14481.867	54.014	***
Error	36	9652.000	268.111		
Total	39	53097.600			
A-priori tests:				F	
Cowpeas in tubes vs. glass beads in tubes				53.179	***
Cowpeas in tubes vs. cowpeas in Petri dishes				0.466	N/S
Gls bds in tbs vs. gls bds in Petri dishes				11.656	**

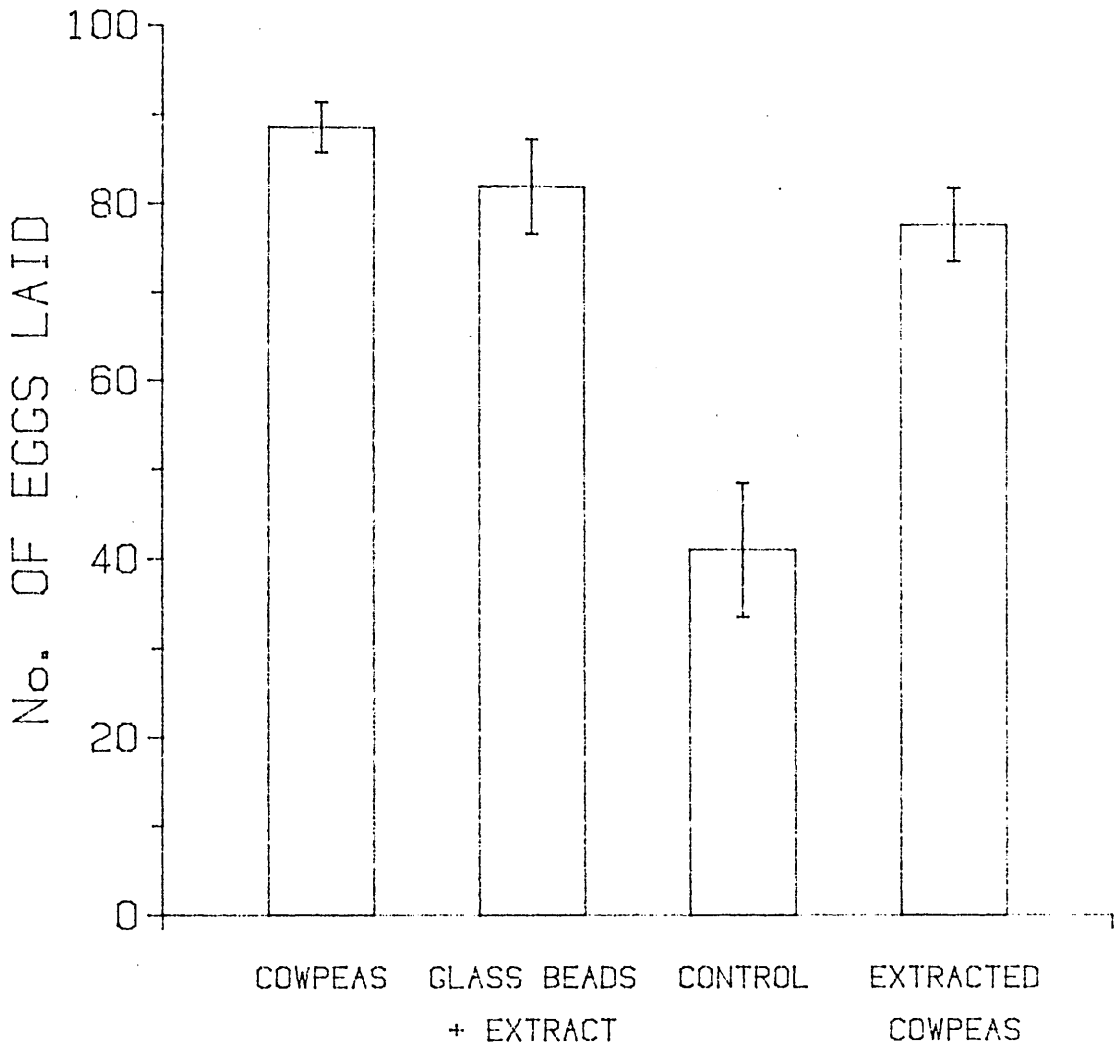


Figure 4.2: Egg laying on glass beads coated with cowpea extract, cowpeas, uncoated glass beads (control) and the cowpeas which the extract was taken from.

Campinas females were mated and then placed onto forty of the appropriate cowpeas or beads. Total egg laying is a mean value from 10 replicates (± 1 S.E.) in which females were allowed to oviposit for 6 days.

Table 4.2: Analysis of variance for egg laying on glass beads coated with cowpea extract and on controls.

Source of variation	DF	SS	MS	F	
Treatments	3	13362.885	4454.295	14.434	***
Error	34	10492.089	308.591		
Total	37	23854.974			
A-priori tests:				F	
Glass beads with extract vs. cowpeas				0.642	N/S
Glass beads with extract vs. glass beads without extract				27.237	***
Cowpeas vs. extracted cowpeas				1.625	N/S

of eggs laid by females on cowpeas using the Mann-Whitney U-test. Eggs laid by the females on the glass beads were more uniformly distributed and this difference was significant ($p < 0.01$).

4.3.3. Different extraction times

Fig. 4.3 shows the mean egg laying and egg production of females on glass beads coated with cowpea extracts collected by soaking cowpeas for different lengths of time (as described in the method). The total number of eggs laid by females on beads coated with the six hour extract was much less than that of females on beads coated with other extracts. Table 4.3 gives the analysis of variance for egg laying results and Table 4.4 gives the same analysis for egg production.

4.3.4. Different concentrations of 10 minute and 24 hour cowpea extracts

Figs. 4.4 and 4.5 show the mean egg laying and egg production of females on glass beads coated with different concentrations of 10 minute and 24 hour cowpea extracts. Table 4.5 gives the analysis of variance of the egg laying results for the 10 minute extract and Table 4.6 gives the same analysis for egg production with the 10 minute extract. Tables 4.7 and 4.8 give the corresponding results for the 24 hour extract.

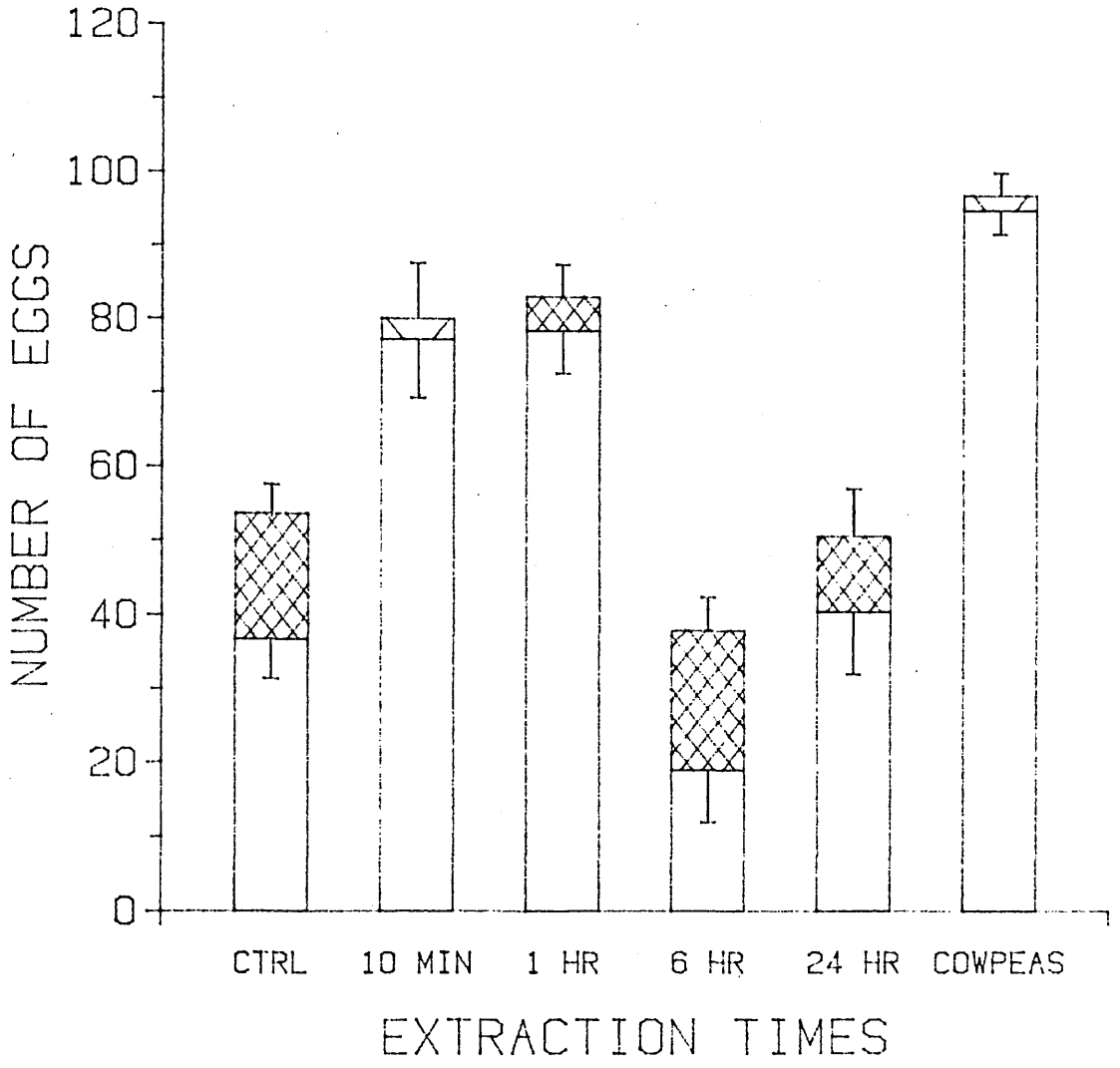


Figure 4.3: Egg laying and egg production on glass beads coated with cowpea extracts which were collected by washing cowpeas for different lengths of time.

Campinas females were mated and placed on forty of the appropriate glass beads or cowpeas and allowed to oviposit for 6 days. Totals are mean values for 10 replicates. The unshaded area of each bar represents total egg laying whilst the shaded and unshaded areas together represent egg production - values given are $\pm$ 1 S.E., the upper error bars are for egg production and the lower error bars are for egg laying.

Table 4.3: Analysis of variance for egg laying on glass beads coated with different cowpea extracts.

Source of variation	DF	SS	MS	F	
Treatments	5	39504.203	7900.841	17.385	***
Error	50	22722.922	454.458		
Total	55	62227.125			
A-priori tests:				F	
Control vs. extracts				6.470	N/S
Amongst extracts				15.454	**

Table 4.4: Analysis of variance for egg production on glass beads coated with different cowpea extracts.

Source of variation	DF	SS	MS	F	
Treatments	5	23455.284	4691.057	15.707	***
Error	50	14932.556	298.651		
Total	55	38387.839			
A-priori tests:				F	
Control vs. extracts				3.145	N/S
Amongst extracts				13.819	**

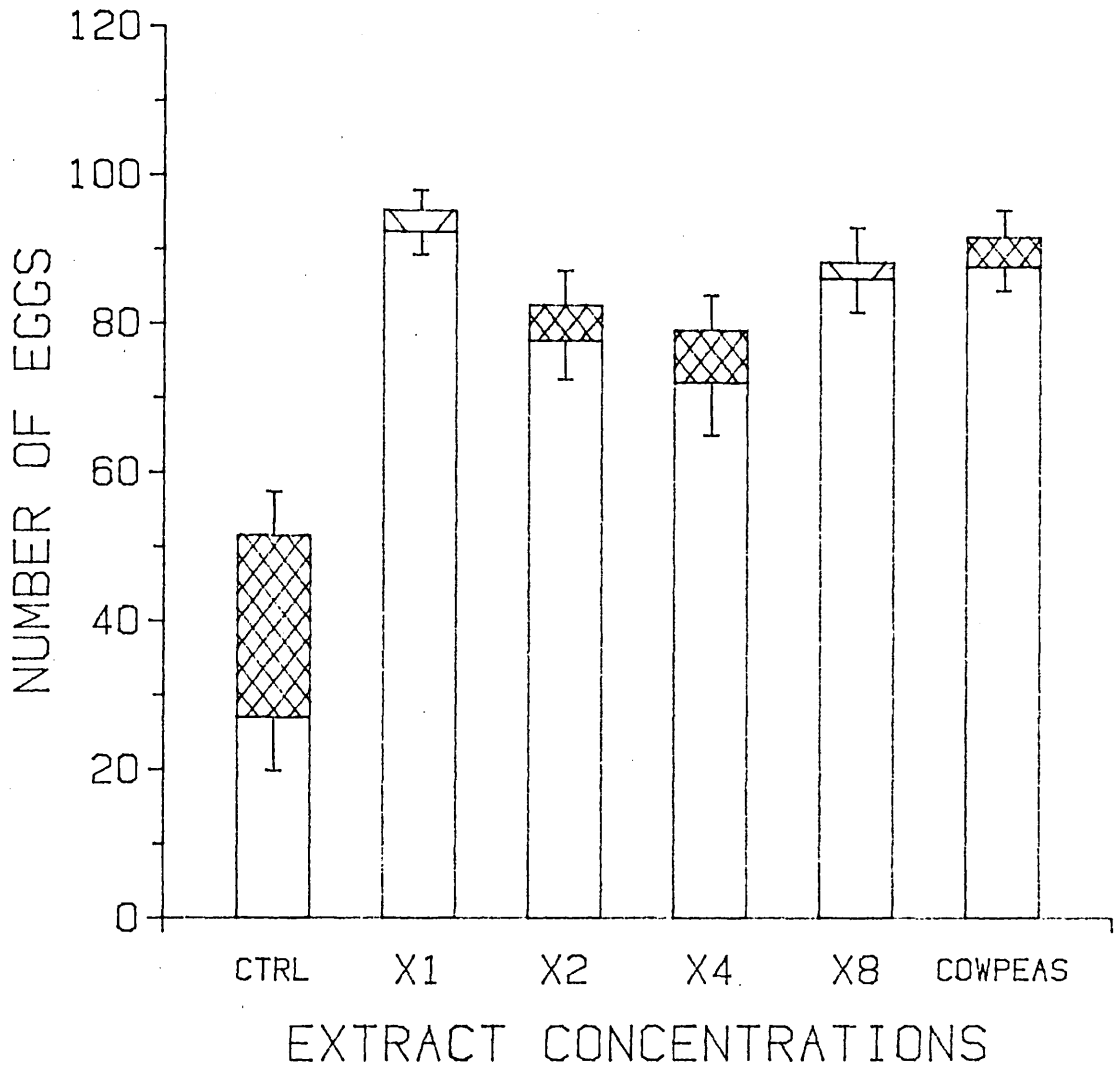


Figure 4.4: Egg laying and egg production on glass beads coated with different concentrations of a '10 minute' cowpea extract.

Campinas females were mated and placed on forty of the appropriate glassbeads or cowpeas and allowed to oviposit for 6 days. Totals are mean values for 10 replicates. The unshaded area of each bar represents total egg laying whilst the shaded and unshaded areas together represent egg production - values given are $\pm$ 1 S.E., the upper error bars are for egg production and the lower error bars are for egg laying.

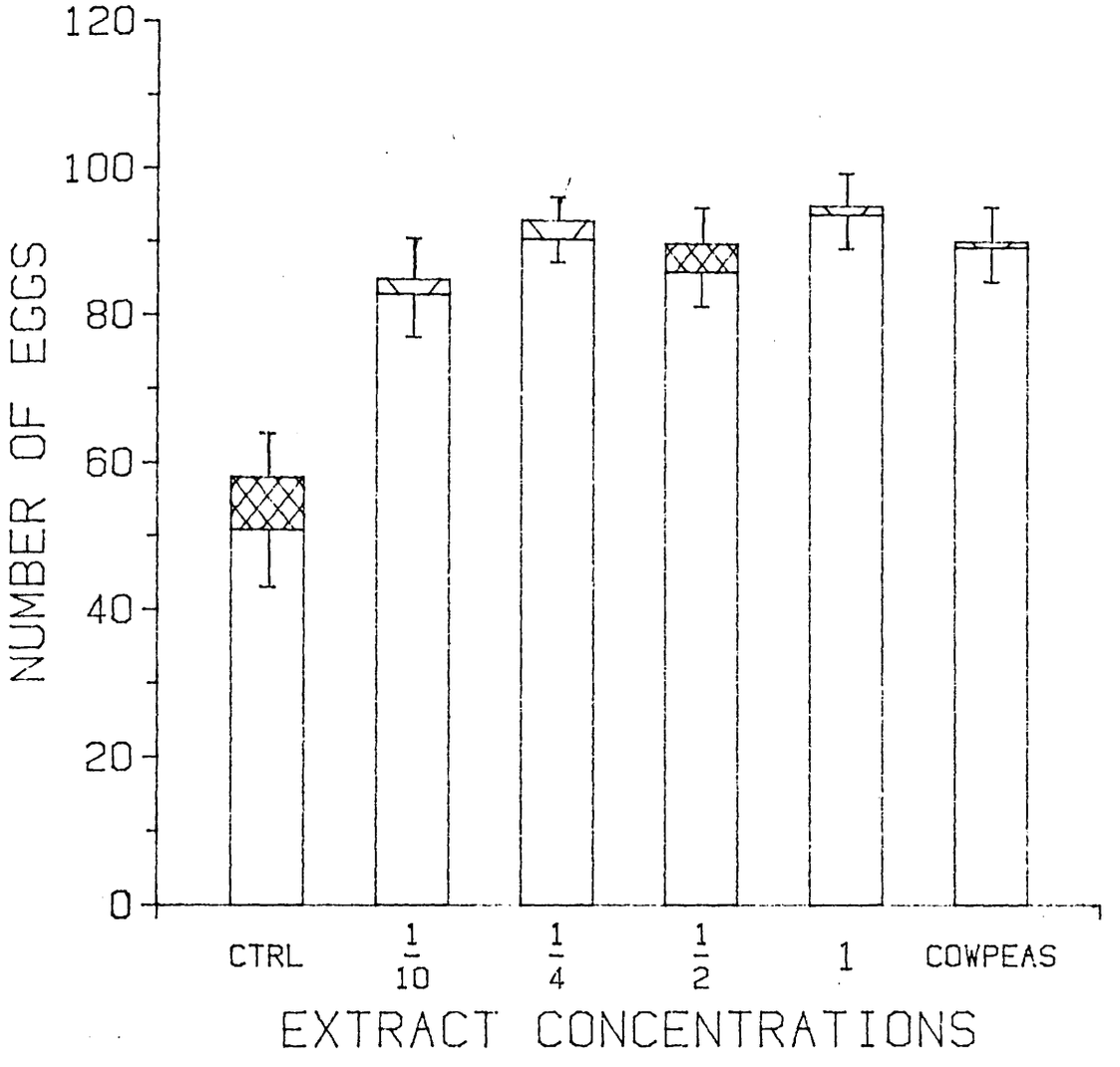


Figure 4.5: Egg laying and egg production on glass beads coated with different concentrations of a '24 hour' cowpea extract.

Campinas females were mated and placed on forty of the appropriate glass beads or cowpeas and allowed to oviposit for 6 days. Totals are mean values for 10 replicates. The unshaded area of each bar represents total egg laying whilst the shaded and unshaded areas together represent egg production - values given are $\pm$ 1 S.E., the upper error bars are for egg production and the lower error bars are for egg laying.

Table. 4.5: Analysis of variance for egg laying on glass beads coated with different concentrations of '10 minute' cowpea extract.

Source of variation	DF	SS	MS	F	
Treatments	5	133.663	26.733	18.520	***
Error	49	70.728	1.443		
Total	54	204.391			
A-priori tests:				F	
Glass beads without extract vs. 10 minute extracts				80.303	***
Among 10 minute extracts				1.923	N/S
1x vs. 8x 10 minute extracts				0.345	N/S

Table 4.6: Analysis of variance for egg production on glass beads coated with different concentrations of '10 Minute' cowpea extract.

Source of variation	DF	SS	MS	F	
Treatments	5	10488.494	2097.699	10.155	***
Error	49	10121.433	206.560		
Total	54	20609.927			
A-priori tests:				F	
Glass beads without extract vs. 10 minute extracts				38.840	***
Among 10 minute extracts				2.247	N/S
1x vs. 8x 10 minute extracts				0.968	N/S

Table 4.7: Analysis of variance for egg laying on glass beads coated with different concentrations of '24 hour' cowpea extract.

Source of variation	DF	SS	MS	F	
Treatments	5	48.414	9.683	8.591	***
Error	50	56.354	1.127		
Total	55	104.768			
A-priori tests:				F	
Glass beads without extract vs. 24 hour extracts				38.909	***
Among 24 hour extracts				0.666	N/S
1/10x vs. 1x 24 hour extracts				1.723	N/S

Table 4.8: Analysis of variance for egg production on glass beads coated with different concentrations of '24 hour' cowpea extract.

Source of variation	DF	SS	MS	F	
Treatments	5	8678.081	1735.616	7.124	***
Error	49	11937.119	243.615		
Total	54	20615.200			
A-priori tests:				F	
Glass beads without extract vs. 24 hour extracts				31.990	***
Among 24 hour extracts				0.819	N/S
1/10x vs. 1x 24 hour extracts				2.162	N/S

4.4. Discussion

Many studies have been made of factors which stimulate oogenesis and oviposition in bruchids. Mating plays a major part in stimulating oogenesis but does not induce oviposition on its own except in extreme circumstances (Ouedraogo & Huignard, 1981 - C. maculatus; Huignard, 1979 - A. obtectus; Pimbert & Pierre, 1983 - Zabrotes subfasciatus). Similarly, the presence of host-plant seeds, whilst stimulating oogenesis, does not, on its own, induce oviposition in C. maculatus (Ouedraogo & Huignard, 1981).

Generally in the Bruchidae, for oogenesis and oviposition to occur, both mating and host-plants are required. In experimental conditions a host-plant extract can be substituted for actual seeds (Applebaum, Gestetner & Birk, 1965; Gokhale & Srivistava, 1973; Monge, 1983). The interaction between mating and host-plants is probably complex and the effects may be synergistic (Pimbert & Pierre, 1983). The necessity of such interaction in the Bruchidae has obvious advantages since both mating and host-plants are prerequisites to the successful reproduction of these insects.

In view of the results of these other studies it was not surprising that C. maculatus females on glass beads laid significantly fewer eggs than females on cowpeas. This means that glass beads alone are not comparable with cowpeas as an oviposition substrate.

Coating glass beads with a cowpea extract provides the otherwise absent host-plant stimulus and allows oviposition to proceed normally. There was no significant difference in the number of eggs laid by females on glass beads coated with cowpea extract and by females on cowpeas. The distribution of eggs on the coated glass beads was significantly more uniform than that of eggs on cowpeas indicating that glass beads are less variable than cowpeas. This demonstrates that glass beads coated with a cowpea extract would be suitable as an artificial oviposition substrate, however, the preparation of the glass beads in this way does take time and so their advantages above cowpeas are limited.

There were slightly fewer eggs laid on the extracted cowpeas than on ordinary cowpeas but this was not statistically significant. The small difference may be due to residue from the acetone, not present on the ordinary cowpeas. Despite the removal of some substances by extraction, the cowpeas used for preparation of extracts still stimulated oviposition in C. maculatus females (Fig. 4.2).

The spatial arrangement of cowpeas did not appear to influence egg laying. There was no significant difference in the number of eggs laid by females on cowpeas in tubes and on cowpeas in Petri dishes. Nor was there any significant difference in the distribution of eggs on the cowpeas in the two arrangements as measured

by the ratio of mean crowding to the mean. Arranging the cowpeas in a single layer did not adversely affect egg laying, neither did it benefit females by allowing them to move more easily between cowpeas (Fig. 4.1). Females on cowpeas stacked in tubes were, apparently, not greatly hampered by having to climb amongst them.

Egg laying on plain glass beads (those without an extract) was significantly higher when the beads were in tubes than when they were in a single layer. This may have been because glass beads in tubes are more stable for females when ovipositing or because females more frequently came into an 'oviposition posture" on the beads, by virtue of being in close contact with them at all times, and laid eggs (albeit very few eggs) despite the lack of host plant stimulation.

Although the cowpea extract collected in the first experiment was prepared by washing the cowpeas for 24 hours it seemed likely, in view of the results of Monge (1983), that an effective extract could be obtained by washing cowpeas for shorter lengths of time.

None of the cowpea extracts (including the 24 hour extract) which were collected by washing cowpeas for different lengths of time, stimulated egg laying to levels as great as that on cowpeas (Fig. 4.3). However, the females on glass beads with the 10 minute extract laid, on average, twice as many eggs as those females on the control.

Conversely, the extract obtained by washing cowpeas in acetone for 6 hours or more did not appear to be effective in stimulating oviposition. Less eggs were laid on the glass beads with the 6 hour extract than on the control, whilst the number of eggs produced was about the same. Thus, there was little stimulation of egg production by the 6 hour extract, which was similar to the control glass beads, and moreover the extract appears to have inhibited oviposition.

The glass beads coated with 6 hour extract or 24 hour extract were noticeably more greasy than the other glass beads. Messina & Renwick (1983) noted that females of C. maculatus avoided ovipositing on beans lightly coated with various oils and suggested that this was physical rather than chemical inhibition. It may be that the 6 hour and 24 hour cowpea extracts presented this physical deterrent.

An alternative explanation for the observed results is that some substance (or substances) which chemically inhibited oviposition was extracted after 6 hours but not after 1 hour. Yet the original host-plant extract experiment showed a 24 hour extract to be effective in stimulating oogenesis and oviposition. In the latter case, however, the evaporation process was incomplete and some of the extract was poured off.

Although differences between egg laying on beads coated with the different cowpea extracts were not statistically significant the results lead to speculation over the effects of different concentrations of the host-plant extracts. In particular, do the 6 hour and 24 hour extracts actually inhibit oviposition and oogenesis and would greater concentration of 10 minute extract inhibit oviposition or oogenesis or both? These questions were the subject of the next group of host-plant extract experiments.

For both types of cowpea extract (10 minute and 24 hour) the results were largely the same. Egg laying by females on glass beads coated with cowpea extract, whether 10 minute or 24 hour extract, was significantly greater than that of females on the respective control beads.

There was no significant difference in egg laying among groups of females placed on beads bearing different concentrations of the same extract (either 10 minute or 24 hour extract), nor was there any difference in the numbers of eggs laid by females on the beads with the highest and lowest concentrations of a given extract (Figs. 4.4 and 4.5).

These results also applied to egg production; there was a significant difference between control and extract groups but no significant difference among the extract groups. Nor was there a significant difference between

the extract groups of the highest and lowest concentrations.

Although egg laying by females on glass beads treated with 10 minute and 24 hour extracts are not strictly comparable, since the females were collected from different cultures, the results do seem similar. Certainly the differences between egg laying on the two different extracts were far smaller than those observed in the previous experiment. It may be that the reduced egg laying by females on beads coated with 6 or 24 hour extract in the previous experiment was due to some experimental error, possibly in the preparation of the extract or in its application to the beads.

There was no evidence of suppression of either oogenesis or oviposition by an increased concentration of the 10 minute extract as was suspected might occur from the results of the previous experiment. The main conclusion of the experiment is that an effective host-plant extract can be obtained by washing cowpeas in acetone for just 10 minutes rather than 24 hours.

The similarity in effect between different concentrations of the same extract indicated that the cowpea extracts did not stimulate oogenesis or oviposition beyond a certain level. It may be that the extracts have a "maximum threshold" of stimulation (exceeded in every case) beyond which they do not stimulate increased oviposition.

Below this supposed "maximum threshold" a reduction in the concentration of the extract might to some degree reduce oogenesis or oviposition proportionally with concentration. In this case all the concentrations studied seem to have allowed maximal egg production; the maximum being judged as egg laying and production by the females on cowpeas.

Alternatively the cowpea extract might merely signify to the female the presence of a suitable host for its eggs; its fecundity and disposal of eggs being governed by other factors (such as adult weight and the presence of other eggs).

It should be remembered that these females had no other substrate to lay their eggs on; if they were given the choice between glass beads with cowpea extract and cowpeas, then the effectiveness of the extract might have been more stringently tested. However, the purpose of the experiment was simply to demonstrate the problems involved in substituting glass beads for cowpeas in other experiments. The presence of host-plant factors must be taken into account when other aspects of the reproductive physiology of C. maculatus are being studied.

Chapter 5

MALE EFFECTS ON FEMALE FECUNDITY

5.1. Introduction

The role of the male insect in reproduction is primarily to ensure the transfer of viable gametes to the female. The secretory products of the accessory glands maintain the spermatozoa during this transfer but may have many other functions (Leopold, 1976). Males of several species of insects contribute to the fecundity of females which use seminal secretions as a source of nutrients (Landa, 1960; Hinton, 1974).

Huignard (1983) demonstrated that females of A. obtectus took up labelled amino acids from spermatophores into their haemolymph and that these were subsequently incorporated into developing oocytes. Material extracted from spermatophores of A. obtectus has also been shown to stimulate oogenesis (Huignard, 1975).

In view of the contribution of the spermatophore to female fecundity in some species of insects it was decided to study the effect of allowing C. maculatus females to remate. Comparing the egg laying of females which had mated only once with that of females which had mated twice might show if extra spermatophore material enabled a female to lay more eggs. The influence of remating on egg hatching could also be observed.

Utida (1941a), Yoshida (1961), Bellows (1982a,b) and Giga (1982) showed that increasing adult density caused a decrease in the fecundity of females of C. chinensis, C. maculatus and C. rhodesianus. In density experiments it is difficult to isolate the effects of adult density from those of egg and larval density. One way of increasing adult density without increasing egg or larval density is to use a fixed number of females and vary the number of males. Males and females are different in their behaviour so changing the density of males alone is not strictly the same as changing the density of all adults. However, such a method does prevent egg density from interfering with the results as there is only a finite number of eggs that a few females can produce and lay.

In addition, Utida (1941b) working on C. chinensis suggested that males caused a reduction in egg laying by disturbing females as they were trying to oviposit and also reduced egg hatching by trampling eggs (though in this case he did not adequately discount the ovicidal effect of any oviposition marker that might have been present). It was of interest to see if such phenomena could also be demonstrated in C. maculatus.

5.2. Method

5.2.1. Effect of remating females

Forty newly emerged Campinas females were allowed to mate and were then placed, without the male, into separate glass tubes containing 40 conditioned cowpeas. These tubes were then placed in the CTH room.

After three days, half way through the major part of the normal oviposition period, half of the females were allowed to remate with newly emerged males. In the event, a total of sixteen females were remated and the remaining four discarded for practical reasons. After remating the females were replaced in their respective glass tubes, returned to the CTH room, and allowed to oviposit until death.

The remaining twenty females were not remated but were removed from their tubes for a short while to compensate for the disturbance of the females which were remated. These remaining females were also returned to the CTH room and allowed to oviposit until death.

Eighteen days after the beginning of the experiment the dead females were removed and the eggs on the cowpeas counted. The number of these eggs which had hatched was also noted.

5.2.2. Different numbers of males

Forty newly emerged Campinas females were collected and allowed to mate. Ten of these females placed in tubes without a male and ten were isolated with the male with which they had originally mated. Of the remaining twenty females, ten were isolated with 5 males each and ten were isolated with 10 males each. Each tube contained forty conditioned cowpeas.

The forty tubes were placed in the CTH room and the beetles left undisturbed for six days. After this period all the beetles were removed and dissected to check the sex of each individual. Any replicate in which a beetle had been wrongly sexed was discarded (this happened in the case of five replicates).

The eggs laid on the cowpeas were counted and the number of those which had hatched was noted.

5.3. Results

Females readily mated a second time. There was no noticeable difference between their readiness to mate and that of newly emerged virgin females.

The numbers of eggs laid by females which mated only once and by females which mated twice are shown in Fig. 5.1. An analysis of variance of these results is shown in Table 5.1.

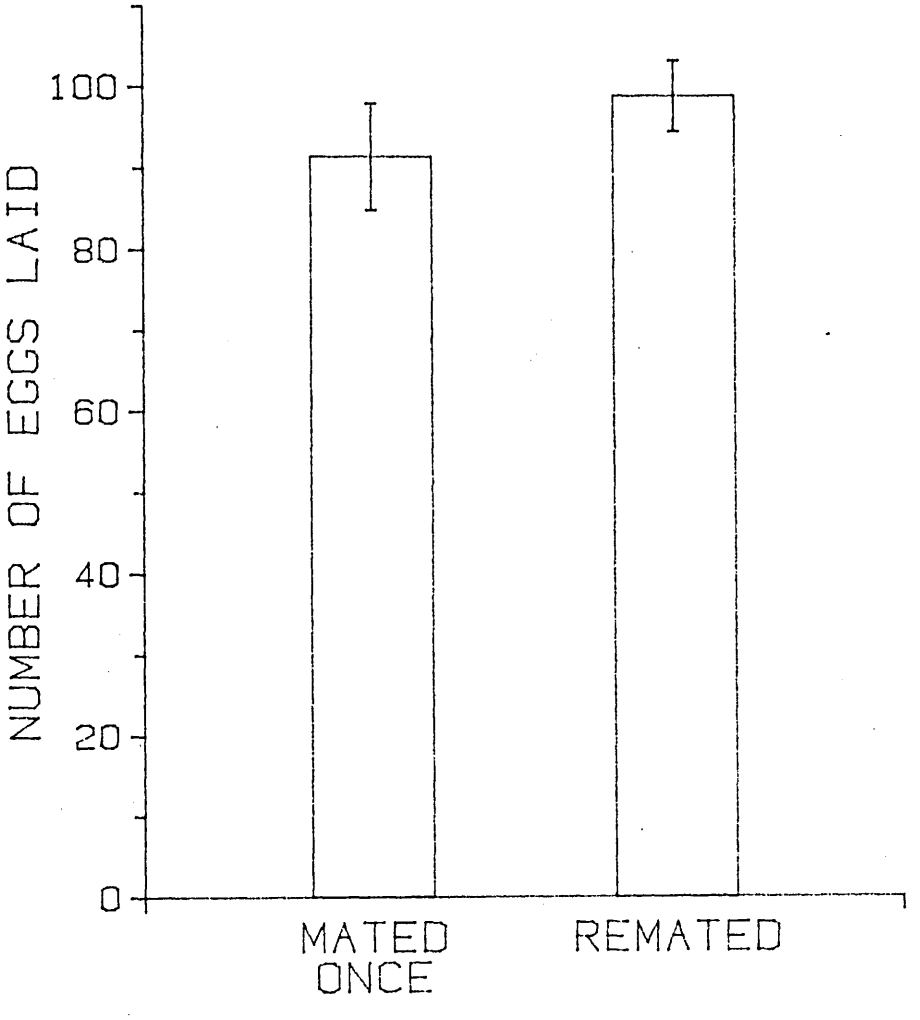


Figure 5.1: The effect of remating.

Mean number of eggs laid (± 1 S.E.) by twenty Campinas females allowed to mate only once before each was placed on forty cowpeas and by twenty Campinas females which were allowed to mate once before each was placed on forty cowpeas but were remated after three days. All females were allowed to oviposit for six days.

Table 5.1: Analysis of variance of egg laying by females mated once or mated twice.

Source of variation	DF	SS	MS	F	
Treatments	1	500.592	500.592	0.847	N/S
Error	32	18906.938	590.842		
Total	33	19407.529			

The hatching of eggs laid by females which mated only once was compared, using a Mann-Whitney U-test, with that of eggs laid by females which were remated. The difference in hatching was not significant.

The numbers of eggs laid by females enclosed with different numbers of males after mating are shown in Fig. 5.2. An analysis of variance was carried out and the results of this are given, along with those of three a-priori tests, in Table 5.2.

For females enclosed with different numbers of males the percentage hatching of eggs and the ratio of mean crowding of eggs on cowpeas to the mean number of eggs per cowpea are shown in Table 5.3. Egg hatching values were compared using the Mann-Whitney U-test. The only significant difference observed was between hatching of eggs laid by females with no males and eggs laid by females isolated with 10 males ($p < 0.05$). The ratio of mean crowding of eggs to the mean number of eggs per cowpea was also compared using the Mann-Whitney U-test. The only significant difference observed was between the distribution of eggs laid by females without males and that of eggs laid by females enclosed with 10 males each ($p < 0.05$).

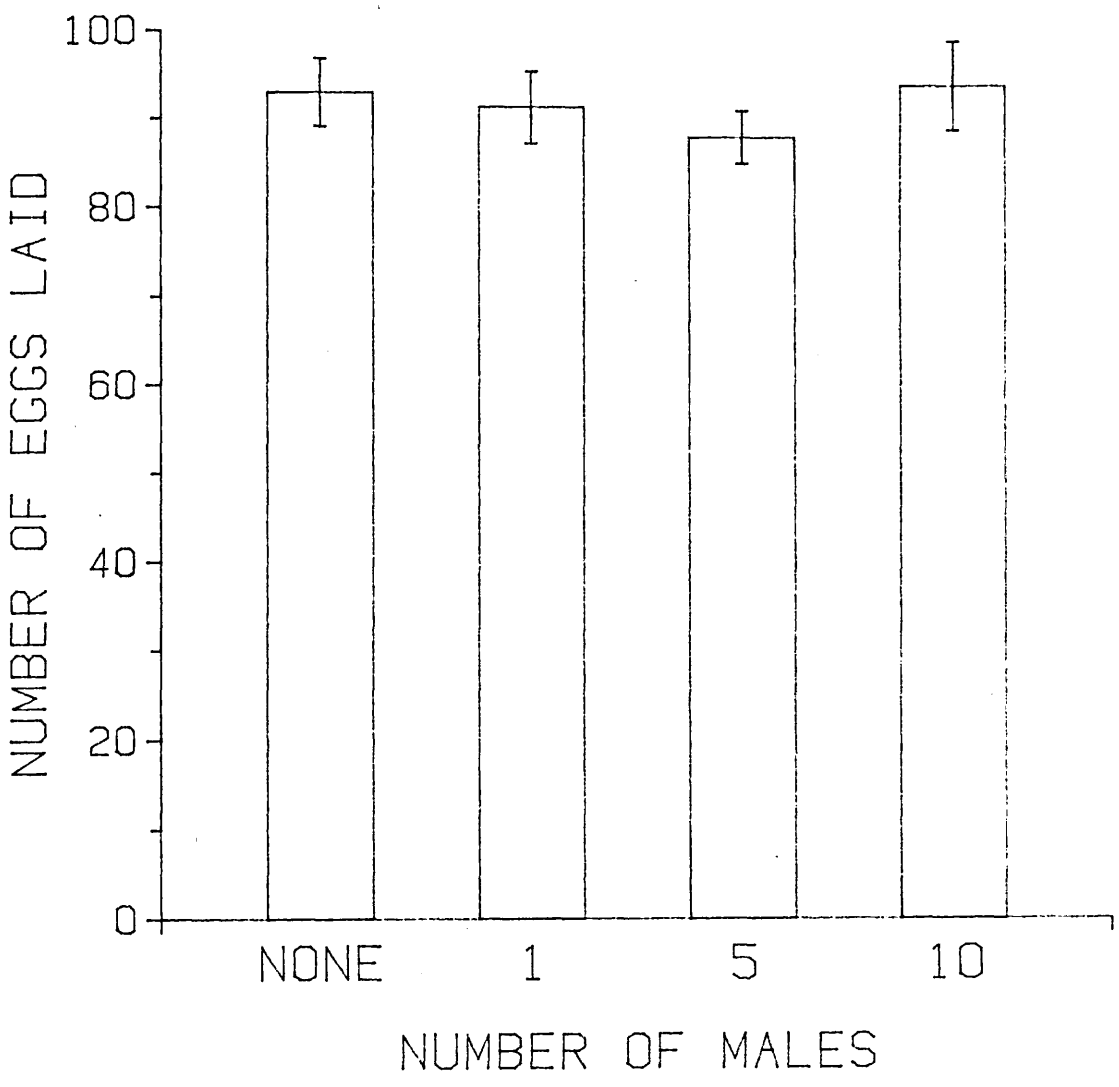


Figure 5.2: The effect of different numbers of males.

Newly emerged Campinas females were allowed to mate and then each was isolated with a different number of males on forty cowpeas for six days. Total egg laying is the mean of ten replicates (± 1 S.E.).

Table 5.2: Analysis of variance of egg laying by females enclosed with different numbers of males.

Source of variation	DF	SS	MS	F	
Treatments	3	351.749	117.250	0.373	N/S
Error	68	21373.362	314.314		
Total	71	21725.111			
A-priori tests:				F	
Female + no male vs. female + 1 male				0.080	N/S
Female + 1 male vs. female + 5 males				0.324	N/S
Female + 1 male vs. female + 10 males				0.189	N/S

Table 5.3: Hatching and ratio of mean crowding to mean of eggs laid by females enclosed with different numbers of males.

Number of males	none	1	5	10
% eggs hatching	92.21	91.21	90.05	90.18
Ratio of mean crowding to the mean number of eggs per cowpea	0.979	1.029	1.038	1.074

5.4. Discussion

Mating is important in egg maturation and oviposition of many species of insects (Englemann, 1970). Its role in oogenesis has been demonstrated in C. maculatus (Ouedraogo & Huignard, 1981) and other bruchids (Huignard, 1979; Pimbert & Pierre, 1983).

Huignard (1968) showed that the number of eggs laid by females of A. obtectus and the fertility of these eggs decreased if copulation was stopped artificially before the time when the pair would normally have parted. One reason for this may be that there is a relationship between the amount of spermatophore material transferred to a female and the number and fertility of eggs laid. Huignard (1983) showed that in A. obtectus spermatophore material is incorporated into oocytes, therefore a link between spermatophore transfer and fecundity in the bruchids seems plausible.

However, the present study showed no significant difference between the total number of eggs laid and egg hatching in C. maculatus females mated once or mated a second time, half way through the oviposition period. Whilst the transfer of the spermatophore stimulates oogenesis and is essential for fertilising eggs these results indicate that the value of more than one spermatophore as food material is minimal and contributes little to the number of eggs laid. A single mating also appeared sufficient to ensure a normal level of egg

hatching.

The effect of population density on fecundity is different in different species of insects. Crowding of adults may either increase or decrease egg laying (Englemann, 1970). Increasing adult density has been shown to cause a decrease in the fecundity of C. chinensis, C. maculatus and C. rhodesianus (Utida, 1941a; Yoshida, 1961; Bellows, 1982a+b; and Giga, 1982).

Utida (1941b) suggested that the reduction in egg laying was caused by adults interfering with each other, especially males attempting to mate with females which were trying to oviposit.

However, the adult densities used in previous studies to demonstrate this reduction in female fecundity were very high. For instance Bellows (1982a) recorded a marked reduction in the number of eggs laid per C. maculatus female at densities of 40 adults (or more) on 3.7 g (about 14-16 seeds) of cowpeas. Such levels of adult crowding are far in excess of those usually found in storage environments (Dobie, pers. comm.) and whilst such high adult densities are perfectly justified when developing models for laboratory populations they have limited relevance to the biology of C. maculatus in the field.

In the present study, females enclosed with 10 males on 40 cowpeas did not lay significantly less eggs than females alone on 40 cowpeas. Males did not prevent females from laying their full complement of eggs.

Whilst not causing a reduction in egg laying males did affect the way females distributed their eggs and the hatching of these eggs. As the number of males increased so the distribution of eggs changed from being more uniform than random to being more aggregated than random. There was a significant difference between the distribution of eggs laid by females without a male and that of eggs laid by females with 10 males. A large number of males appeared to disrupt the normal oviposition behaviour of the females and caused them to aggregate their eggs slightly.

The changes in egg distribution appear so slight, however, and the densities of eggs per cowpea were so low, that such small variations in the distribution of eggs would be unlikely to have a great affect upon the number of adults subsequently emerging.

Utida (1941b), working on C. chinensis, found that the numbers of eggs which hatched decreased with increasing adult density, Bellows (1982a) also demonstrated this for C. maculatus. The greater part of these decreases occurred at very high adult densities (Utida (1941b) - 256 adults on 112 azuki beans; Bellows (1982a) - 40 adults (or more) on 14-16 cowpeas).

In the present study a significant difference was found between the hatching of eggs laid by females alone and those laid by females enclosed with 10 males. However, even though statistically significant, the difference in the mean percentage of eggs hatched was only 2.03 % (Table 5.3).

These results indicate that males can interfere with the egg laying of females but whether this interference is caused by physical contact between males and females, by adults trampling eggs, or by chemical substances deposited by the males remains unclear. To separate such influences would be extremely difficult.

Although adult density has been shown to have a significant influence upon many aspects of the population dynamics of C. maculatus, because of the low adult densities generally found in nature such influences will rarely be of importance. The extreme larval densities, which are often found in stored cowpeas (Dobie, pers. comm.), are of far greater significance.

Chapter 6

THE OVIPOSITION DETERRING PHEROMONE OF *Callosobruchus maculatus*

6.1. Presence of the egg marker

6.1.1. Introduction

The marking of eggs or egg laying substrates with chemicals which deter subsequent oviposition in the vicinity has been described for several species of insects (Price, 1970; Prokopy, Reissig and Moericke, 1976; Zimmerman, 1979; Ditterick, Jones & Chiang, 1983; Renwick & Radke, 1983). In the Bruchidae oviposition markers have been demonstrated for *Callosobruchus chinensis* (Honda, Oshima & Yamamoto, 1976) and *Acanthoscelides obtectus* (Szentesi, 1981). There is also strong circumstantial and experimental evidence for the existence of a chemical marker produced by *C. maculatus* (Mitchell, 1975; Wasserman, 1981; Giga, 1982) and by *C. rhodesianus* (Giga, 1982).

The following section describes experiments designed to demonstrate the existence of an oviposition marker for *C. maculatus* and the design of a bioassay to investigate its properties.

6.1.2. Materials and methods

Experimental Equipment

Choice chambers were made from plastic Petri dishes (Sterilin Ltd, Teddington, England) which were 8.5 cm in diameter by 1.3 cm deep. These Petri dishes were separated into quadrants using Perspex (ICI Ltd., London) dividers. The dividers were notched along the edges to allow beetles to pass freely from one sector to another whilst preventing the cowpeas from mixing. The plastic dividers were held in place using plastic cement ("Tensol" Cement No. 6, ICI Ltd., London). To allow air to circulate a hole 2 cm in diameter was cut into the lid; this hole was covered by plastic gauze which was held in place by plastic cement. The quadrants of the choice chambers were numbered one to four in a clockwise direction so that quadrant 1 was opposite quadrant 3 and quadrant 2 was opposite quadrant 4. Fig. 6.1 is a photograph of one of these choice chambers.

The choice chambers were easily washed in detergent to remove any traces left by the beetles. Where beetles were isolated for only 24 hours, oviposition on the containers was rare and so they were not lined with emery cloth.

Figure 6.1: A choice chamber

Note the notches in the dividers which allow the beetles free access to each quadrant whilst preventing the beans from mixing.

Preparation of the marker

In a typical experiment four hundred beetles (unsexed) were placed on 500 clean glass beads in a crystallizing dish. The dish was covered with plastic gauze, to prevent the beetles from escaping, and placed in the CTH room. Twenty-four hours later the beetles were removed from the glass beads and discarded.

The eggs which had been laid on fifty glass beads were then counted, care being taken to avoid contamination from dirty hands or surfaces, to calculate the egg density. 250 ml of acetone then was poured onto the beads and after 10 minutes this was decanted. 50 ml of fresh acetone was then used to rinse the beads and was added to the 250 ml already collected. This was the 'egg marker' and was refrigerated (at 4 °C) in a sealed glass container until required. Acetone was used because it is a wide ranging solvent and had been used previously for the cowpea extract experiments (Chapter 4).

The marker was poured onto 400 sieved cowpeas which had been carefully examined to ensure that they had whole, unbroken seedcoats. The acetone was evaporated under reduced pressure, at 30 °C, using a rotary evaporator (a process which usually took about 15 minutes). In this way each cowpea was evenly coated with egg marker.

The relative strength of a particular egg marker could be roughly calculated from the number of eggs on the glass beads and the number of cowpeas onto which the marker was evaporated. Thus, if 1200 eggs (as calculated from the sample) had been laid on the glass beads and the marker which was prepared from these glass beads was evaporated onto 400 cowpeas then each cowpea was held to have been coated with three egg equivalents of marker. This does not mean, however, that a female beetle would react to such a cowpea as it would to a cowpea with three eggs on it because certain components of the marking pheromone may be not be dissolved, some of the marker would have been lost on the sides of the glass containers during the evaporation process and there was no physical presence of eggs; it is merely a way of estimating the strength of the marker.

Control cowpeas were also prepared, in a similar manner, by pouring acetone only onto them and evaporating this off in the rotary evaporator. Marked and control cowpeas were always prepared on the same day.

Marked cowpeas without choice; for 6 days

Egg marker was evaporated onto 400 conditioned cowpeas so that each cowpea was coated with marker equivalent to 3.75 eggs. 400 control cowpeas were also prepared.

40 marked cowpeas were placed in each of ten glass tubes. 40 control cowpeas were placed in each of an additional ten glass tubes and finally ten more tubes were prepared each containing 40 fresh, conditioned but untreated cowpeas (ordinary cowpeas). All three sets of tubes were then placed in the CTH room for three days to allow their temperature and moisture content to stabilise (in later experiments this period was reduced to overnight because the moisture content was not felt to be critical and the production of adults from the cowpeas was not investigated).

A newly emerged and mated Campinas pair was placed in each of the thirty tubes. These beetles were left undisturbed for 6 days before they were removed. A week later the eggs were counted and egg hatching was noted.

Egg laying by females on marked cowpeas only; for 12, 24 and 48 hours

Egg marker was evaporated onto 300 conditioned cowpeas to produce individual cowpeas coated with marker equivalent to 4.4 eggs per cowpea. 300 control cowpeas were also prepared.

10 marked cowpeas were placed in each of 30 glass tubes and 10 control cowpeas were placed in each of a further 30 glass tubes. The 60 tubes were left in the CTH room overnight to allow the temperature of the cowpeas to stabilise. The following morning a newly

emerged and mated Campinas pair was placed on the cowpeas in each of the tubes.

Beetles were removed from ten tubes containing marked cowpeas and from ten tubes containing control cowpeas after 12, 24 and 48 hours. Since the beetles were left on the cowpeas for a maximum of two days, only 10 cowpeas were necessary to prevent suppression of egg laying due to a lack of oviposition sites. The cowpeas were left for a week to allow the eggs to hatch, after which the eggs were counted and hatching noted.

Choice chamber experiment

A control experiment was designed to test whether there was any bias in the choice chambers or method of collecting the egg marker which might affect the results of experiments.

500 glass beads were cleaned as usual with detergent ("Teepol", BDH Chemicals Ltd, Poole, England) and then chromic acid. They were then thoroughly washed in distilled water and dried. 250 ml of acetone was poured onto the beads for ten minutes and then decanted, the beads were then rinsed in a further 50 ml of acetone which was added to the original 250 ml. The acetone was then poured onto 400 cowpeas and evaporated to coat the cowpeas in any residue left by the cleaning process; 400 control cowpeas were also prepared using acetone alone.

10 of the 'residue'-coated cowpeas were placed in each of quadrants 1 and 3 of twenty choice chambers and 10 control cowpeas were placed in each of quadrants 2 and 4 of the same choice chambers. The choice chambers were placed in the CTH room overnight and the following morning a newly emerged and mated Campinas pair was placed in each. The beetles were left for twenty-four hours and then removed. The eggs were counted a week later and hatching noted.

After the control experiment was completed experiments with egg markers were carried out. Egg marker was evaporated onto 400 conditioned cowpeas to give individual cowpeas coated with egg marker equivalent to 3.6 eggs. In addition 400 control (unmarked) cowpeas were prepared.

10 marked cowpeas were placed in each of quadrants 1 and 3 and 10 control cowpeas were placed in each of quadrants 2 and 4. This gave twenty choice chambers offering a choice between 20 marked and 20 control cowpeas. The choice chambers were then placed in the CTH room overnight.

A newly emerged and mated Campinas pair was placed in each of the choice chambers. The beetles were left for 24 hours after which they were removed and discarded. The cowpeas were left for a week to allow the eggs to hatch and then the numbers of eggs on the control and on the marked cowpeas were counted. Egg hatching was also

noted. Where no eggs were laid the result was ignored since this provided no information about a female's choice between the two sets of cowpeas (this happened rarely).

Marked and control cowpeas intermingled

Egg marker was evaporated onto 400 conditioned cowpeas to give individual cowpeas coated with marker equivalent to 2.56 eggs per cowpea. 400 control cowpeas were also prepared.

Individual cowpeas were then labelled with red water-soluble ink. Every marked cowpea was labelled with a short, thin line near they eye of the cowpea and every control cowpea was labelled with a similar short mark near the keel of the cowpea (the terms for positions on the cowpea are those used by Nwanze, Horber & Pitts, 1975). Care was taken to avoid any cross-contamination between marked and control cowpeas during labelling.

Twenty marked and twenty control cowpeas were placed together in each of twenty undivided plastic Petri dishes. The cowpeas were randomly distributed in the Petri dishes so that, for instance, a marked cowpea may have been next to other marked cowpeas or control cowpeas. Because there was plenty of room for forty cowpeas in the Petri dishes they rarely touched but this was not prevented when they did. The Petri dishes were then left in the CTH room overnight.

The following morning a newly emerged and mated Campinas pair was placed in each Petri dish. The beetles were left for twenty-four hours after which they were removed and discarded. The cowpeas were left in the CTH room for one week, the eggs were then counted and egg hatching was noted.

6.1.3. Results

Marked cowpeas without choice for 6 days

The total numbers of eggs laid on the marked cowpeas, the control cowpeas and the ordinary cowpeas are shown in Fig. 6.2. An analysis of variance of these results is shown in Table 6.1 along with an a-priori test between egg laying totals on marked and control cowpeas. There was no significant difference between the numbers of eggs laid on the marked and control cowpeas.

A comparison of the ratio of mean crowding of eggs on control and on marked cowpeas, using the Mann-Whitney U-test, showed a significant difference ($p < 0.01$) in the distribution of eggs on the marked and control cowpeas. The distribution of eggs on the control cowpeas tended towards uniformity (mean crowding/mean = 0.878) whilst that of eggs on the marked cowpeas was slightly aggregated (mean crowding/mean = 1.114).

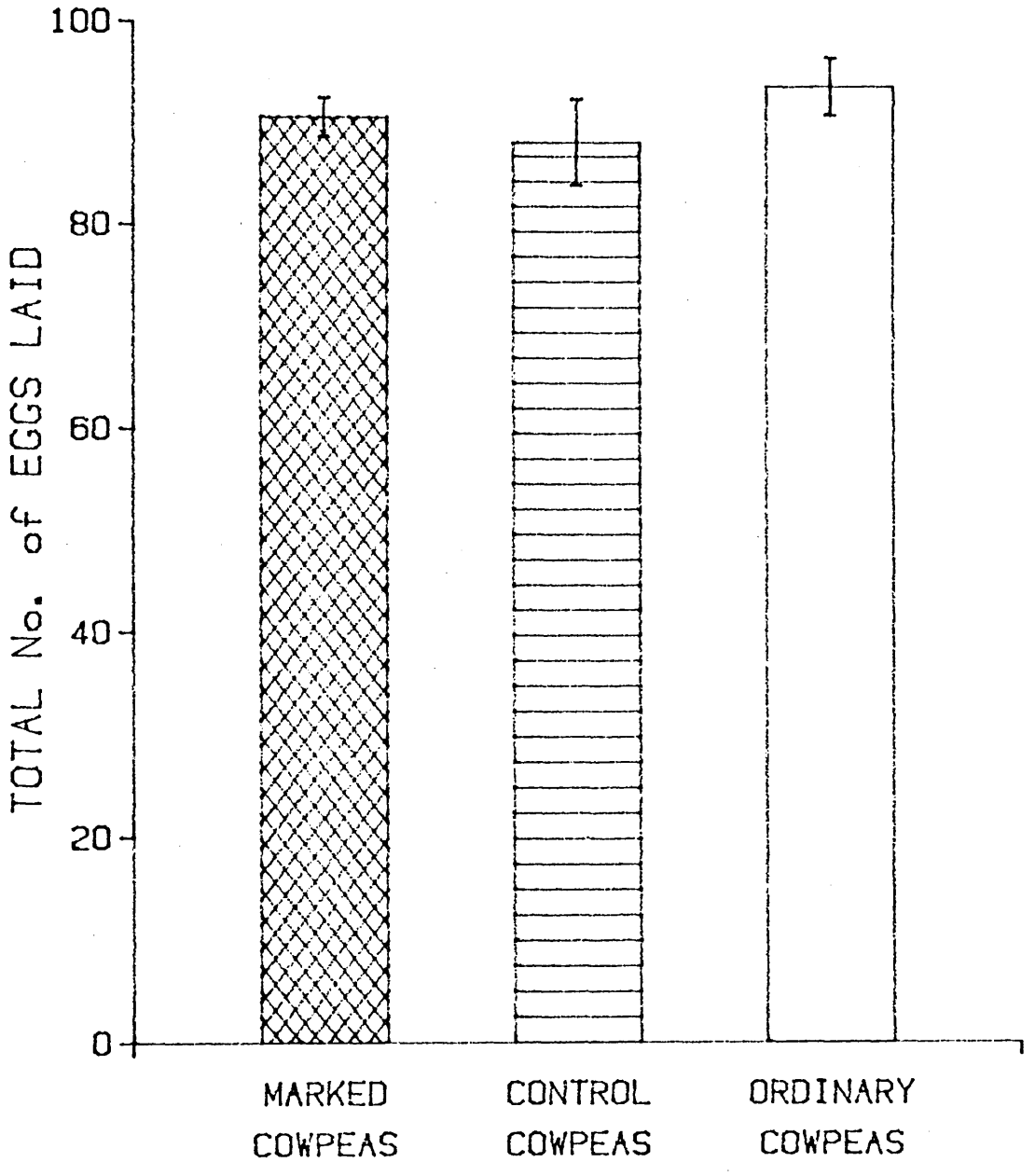


Figure 6.2: Total number of eggs laid over 6 days on marked, unmarked (control) or ordinary cowpeas by females given no choice.

A newly emerged and mated Campinas pair were placed on forty cowpeas of the appropriate type and the female allowed to oviposit for 6 days. Total egg laying is the mean value for ten replicates (± 1 S.E.)

Table 6.1: Analysis of variance of egg laying by females on marked, control or ordinary cowpeas with no choice for 6 days.

Source of variation	DF	SS	MS	F	
Treatments	2	147.755	73.877	0.674	N/S
Error	26	2851.556	109.675		
Total	28	2999.310			
A-priori test:				F	
Control cowpeas vs. marked cowpeas				0.263	N/S

There was no significant difference in the percentage hatching of eggs laid on marked cowpeas (89.7±0.1%) and that of eggs laid on the control cowpeas (79.3±5.5%).

Marked cowpeas without choice for 12, 24 and 48 hours

The total numbers of eggs laid by females on marked or control cowpeas for 12, 24 and 48 hours are shown in Fig. 6.3. Student's t-tests were carried out between the total number of eggs laid by females on control and marked cowpeas for the same length of time but there was no significant difference in any of the three comparisons.

The average ratio of mean crowding to the mean number of eggs per cowpea and the average percentage hatching of eggs on control and marked cowpeas for the 12, 24 and 48 hour experiments are given in Table 6.2.

The distribution of eggs, measured by the ratio of mean crowding to mean number of eggs per cowpea, laid by females on the two types of cowpeas (marked and control) was compared using the Mann-Whitney U-test. There was no significant difference between the distribution of eggs laid by females which were on cowpeas for 12 hours but the distribution of eggs laid by females which were on control cowpeas for 24 or 48 hours was significantly more uniform than that of the eggs laid by females on marked cowpeas for the same time.

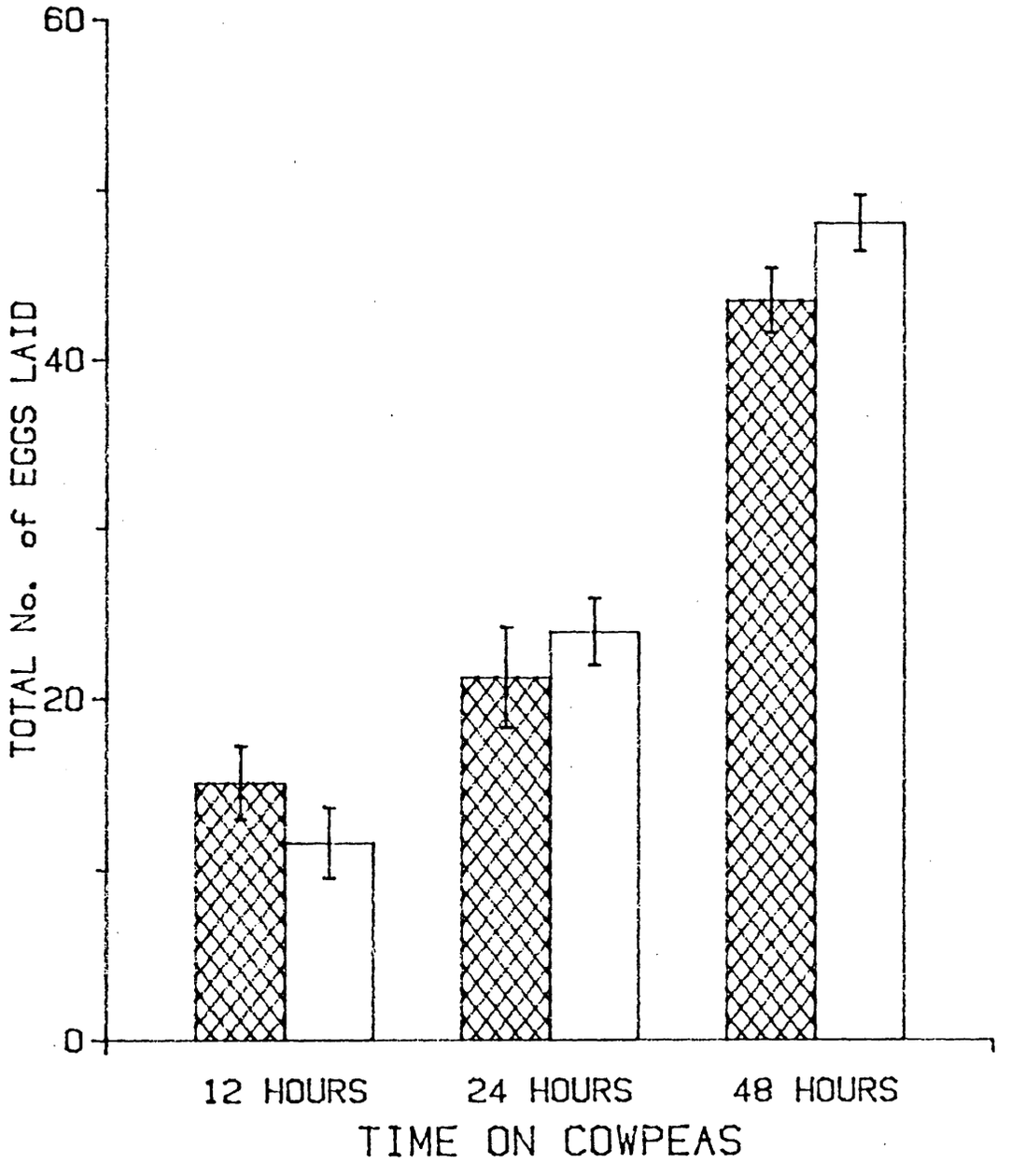


Figure 6.3: Total number of eggs laid over 12, 24 and 48 hours on marked and unmarked (control) cowpeas by females given no choice.

Shaded bars - marked cowpeas
Unshaded bars - control cowpeas

A newly emerged and mated Campinas pair were placed on ten cowpeas of the appropriate type and the female allowed to oviposit for 6 days. Total egg laying is the mean value for 10 replicates (± 1 S.E.)

Table 6.2: Ratio of mean crowding to mean and percentage hatching of eggs laid by females with no choice on marked or control cowpeas over 12, 24 or 48 hours.

Time on cowpeas	Cowpea type		
	control	marked	
Mean crowding/mean			
12 hours	1.235	1.512	N/S
24 hours	0.932	1.137	*
48 hours	0.932	1.072	*
% egg hatching			
12 hours	95.01	89.55	N/S
24 hours	92.42	90.45	N/S
48 hours	92.41	90.88	N/S

There was no significant difference in the hatching of eggs laid by females which were placed on marked or control cowpeas for the same length of time.

Choice chamber experiments

The proportion of eggs laid on the cowpeas coated with any cleaning residue and on the control cowpeas is shown in Fig. 6.4, a Wilcoxon matched-pair test showed no significant difference between the number of eggs laid on the two groups of cowpeas.

The proportion of eggs laid on marked and control cowpeas in choice chambers is also shown in Fig. 6.4. There were significantly more eggs laid on the control cowpeas than on the marked cowpeas ($p < 0.01$).

The proportion of eggs laid on marked and on control cowpeas which were intermingled is shown in Fig. 6.4. As before there were significantly more eggs laid on the control cowpeas than on the marked cowpeas ($p < 0.001$).

6.1.4. Discussion

Honda et al (1976) working with C. chinensis found that the egg laying of females on azuki beans, Phaseolus angularis, was reduced by an oviposition marker. Giga (1982), working with C. maculatus, found a reduction in the egg laying of females placed for three hours onto cowpeas which had five or more eggs deposited

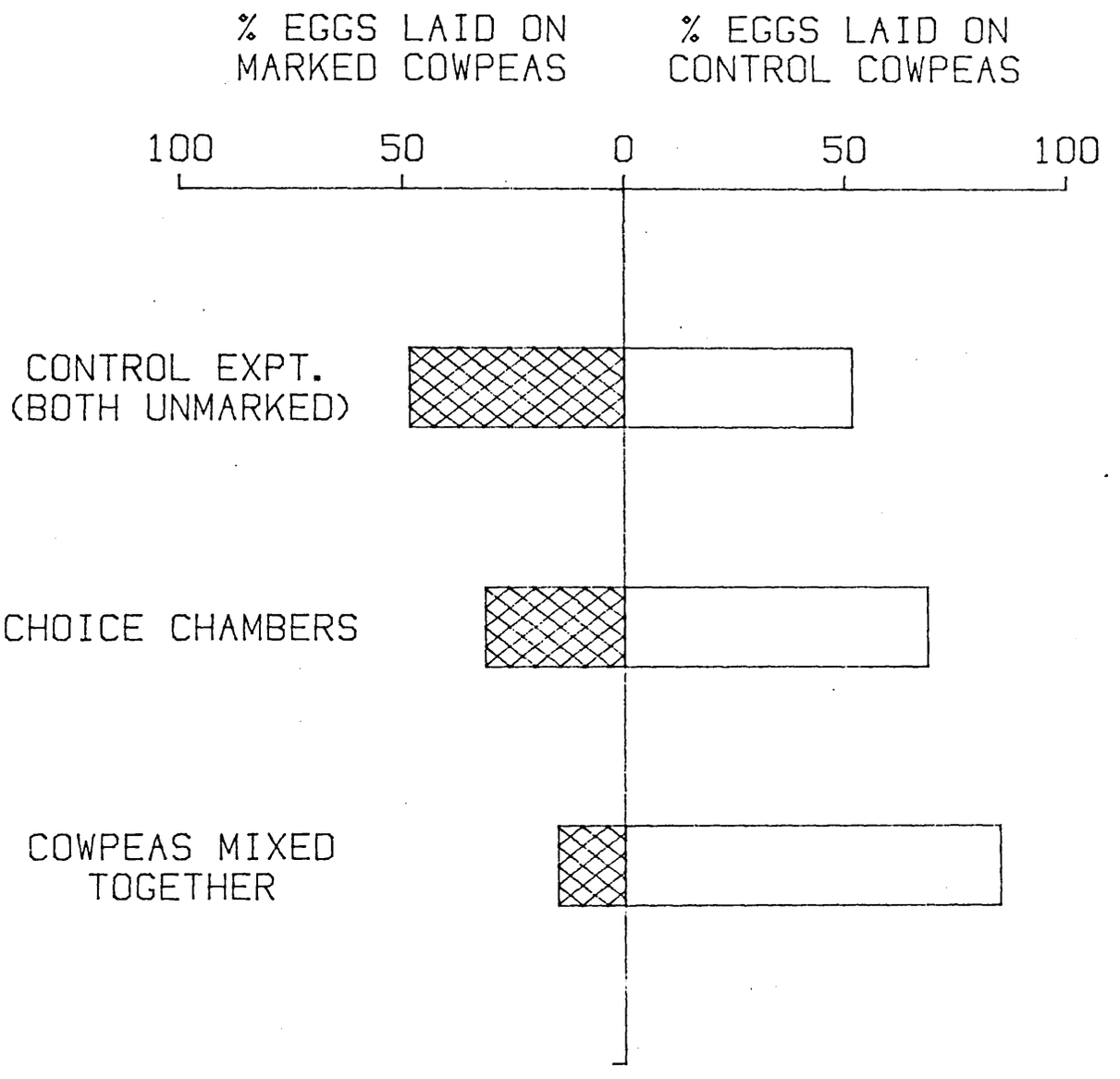


Figure 6.4: Eggs laid on a choice between marked and unmarked (control) cowpeas.

The percentage of eggs (average for twenty replicates) laid over 24 hours on a choice between 20 marked and 20 unmarked (control) cowpeas by newly emerged and mated Campinas females.

on them. In the same study he also found that egg laying of females was reduced when they were placed for ten days on cowpeas conditioned by adult males (but not by virgin adult females). Yoshida (1961) found that the fecundity of C. maculatus was not reduced on conditioned cowpeas but that egg hatching on such cowpeas was reduced. Honda et al (1976) also observed that oviposition marker caused a reduction in hatching of eggs laid by C. chinensis.

The results in the present study contradict, to some extent, the findings of both Yoshida (1961) and Giga (1982). Where females were presented with marked cowpeas only, for six days, there was no reduction in egg laying nor any reduction in egg hatching when compared to the females on control cowpeas. With no choice, females laid their full complement of eggs on marked cowpeas and the eggs survived as well as those laid on control cowpeas. Fig. 6.2 shows that the numbers of eggs laid on the marked and control cowpeas was very similar to the number laid on the ordinary cowpeas indicating that any small amount of solvent left from the evaporation process does not reduce the number of eggs laid.

Placing females on marked cowpeas for shorter lengths of time might have been expected to cause a reduction in egg laying when compared with females on control cowpeas. This is because females on marked cowpeas might delay their egg laying whilst they search for unmarked cowpeas which would provide a better chance

of survival for their offspring. In fact, females on marked cowpeas for 12, 24 or 48 hours did not reduce their egg laying compared with females on control cowpeas for the same length of time.

In this case the marked cowpeas, coated with marker equivalent to 4.4 eggs per cowpea, may not have provided sufficient deterrent to delay the oviposition of the females. Honda et al (1976) obtained a reduction in egg laying of C. chinensis with marker which was roughly equivalent to 30 eggs per bean.

A reduction in egg laying by C. maculatus may have been obtained if females were placed on the cowpeas for an even shorter length of time (thereby explaining the discrepancy of these results with those of Giga, 1982) or if the cowpeas were coated with more marker, but it is apparent that, in the long term, females will readily lay eggs on marked cowpeas when provided with no alternative.

The females on the marked cowpeas did distribute their eggs less uniformly than females on the control cowpeas indicating that the marker disrupted the normal oviposition behaviour of the females. Honda et al (1976) found that, at certain levels, marker material caused C. chinensis females to lay their eggs in a random manner and that if the amount of marker material was further increased the eggs were more aggregated.

In the present study there was a significant difference in the distribution of eggs on marked and control cowpeas after females had been on the cowpeas for twenty-four hours or more. The eggs of females placed on marked cowpeas for 1, 2 and 6 days were slightly aggregated whilst those of females on control cowpeas for the same lengths of time were more uniformly distributed.

The biological significance of such a difference is probably small since the highest density of eggs per seed on the marked cowpeas was 11 per cowpea and this would be unlikely to severely reduce the survival or fecundity of emerging adults. However, the oviposition marker did alter the oviposition behaviour of C. maculatus females making them distribute their eggs differently.

Mitchell (1975), Wasserman (1981) and Giga (1982) postulated that an oviposition marker produced by C. maculatus, for which there was a great deal of evidence, would deter females from laying eggs on cowpeas coated with it. Offering females a choice between marked and control cowpeas is the obvious next step to demonstrate the presence of an oviposition deterrent. This was the method used by Oshima, Honda & Yamamoto (1973) to demonstrate the production of such a marker by C. chinensis and it was also used by Szentesi (1981) working on A. obtectus.

The chambers designed to offer the choice between marked and control cowpeas were first tested to see if there was any bias that might make females lay more eggs in one quadrant than another or if any cleaning residue from the glass beads had an effect. The result of this experiment showed that there was no bias towards particular quadrants and that the way the glass beads were cleaned did not affect the reaction of females to the cowpeas.

Females offered the choice of marked or unmarked (control) cowpeas laid significantly more eggs on the unmarked cowpeas than on the marked cowpeas. In fact, females laid twice as many eggs on the unmarked cowpeas than on the marked cowpeas. The egg marker clearly reduced the number of eggs laid on the marked cowpeas and therefore acted as an oviposition deterrent.

Cowpeas in choice chambers were divided into four lots of ten. By presenting cowpeas in this way the egg marker may be more effective (due to a group effect) than if the marked and control cowpeas were mixed together. Mitchell (1975) presented a model for the oviposition strategy of C. maculatus in which a female compared the cowpea on which it rested with the previous one it visited when deciding whether to oviposit or not (if the present bean had less eggs than the previous one then it would tend to lay an egg). If this hypothesis were true then mixing marked and control cowpeas would make it

easier for females to discriminate between the two types of cowpea and thus lay a greater proportion of eggs on the control cowpeas. In the choice chambers a female would be more likely to compare a marked cowpea with another one and lay eggs on marked cowpeas despite the fact that control cowpeas with less eggs were available.

The result of mixing cowpeas in this way was that females were more strongly deterred from ovipositing on marked cowpeas than were females on marked cowpeas in choice chambers. Females laid about 16% of their eggs on the marked cowpeas compared with 33% laid on marked cowpeas in choice chambers. This result gives greater credence to the hypothesis that C. maculatus females compare one cowpea with another when deciding whether to oviposit or not.

Although mixing cowpeas together did give a greater response than when they were in choice chambers, labelling individual cowpeas was a time consuming process. The choice chambers were effective at demonstrating the effect of the oviposition marker and provided the basis of a convenient bioassay so that the properties of the oviposition marker could be studied. Because of this, choice chambers were used for all later experiments.

6.2. Properties of the marker

6.2.1. Introduction

Having established that the method of collecting the oviposition marker and the bioassay used to test it were both effective, it was possible to investigate the properties of the marker.

Of particular interest is the chemical nature of the marker. To isolate and identify the active components is very complicated but a valuable first step is to discover whether the marker is soluble in various solvents.

The length of time over which the marker remains effective is of practical importance and again gives some indication of the chemical nature of the marker. The marker would be more suitable for control purposes if it were persistent than if it evaporated away quickly. Also, certain chemicals evaporate more quickly than others and this can give an indication of which chemicals make up a pheromone.

Another area of interest is how the strength of the marker affects the reaction of females to it. As the strength of the marker was increased would the proportion of eggs laid on the marked cowpeas decrease or would it stay the same?

6.2.2. Materials and methods

Marker in different solvents

About 800 beetles were placed on 1200 clean glass beads for 48 hours. The beetles were then removed and the glass beads were divided into 3 equal lots. 250 ml of acetone was added to one lot of beads for 10 minutes. This was decanted and the beads were washed in a further 50 ml of acetone which was then added to the original liquid. This gave marker dissolved in 300 ml of acetone.

The process was repeated using Analar grade petroleum ether (boiling point = 40-60 °C) and Analar grade dichloromethane; one solvent for each of the remaining two lots of beads.

The solvents containing the markers were refrigerated at 4 °C for one week. Each marker was evaporated onto separate lots of 400 conditioned cowpeas to give cowpeas coated with marker equivalents of 4.4 eggs per cowpea. In addition 3 lots of 400 control cowpeas were prepared using the appropriate solvents.

10 marked cowpeas (coated with marker which was dissolved in one solvent) were placed in each of quadrants one and three of twenty choice chambers. 10 control cowpeas (prepared using the same solvent as the marked cowpeas) were placed in each of quadrants 2 and 4 of the same twenty choice chambers. This was repeated for all three types of marked and control cowpeas. Thus

there were three sets of twenty chambers offering the choice between 20 marked and 20 control cowpeas prepared using the same solvent.

The choice chambers were placed in the CTH room overnight to allow the temperature of the cowpeas to stabilise. The following morning a newly emerged mated Campinas pair was placed in each choice chamber. The beetles were left for 24 hours before they were removed and discarded. The cowpeas were left for a week to allow the eggs to hatch, after which the eggs were counted and egg hatching noted.

Solubility of marker in water

About 500 beetles were placed on 500 clean glass beads for twenty-four hours. 250 ml of non-organic water (water distilled and filtered through a 45 μm mesh to remove all organic matter such as bacteria) was poured onto the beads. The water was left for thirty minutes at room temperature before it was poured off, the beads were then rinsed in a further 50 ml of non-organic water which was added to the original 250 ml. The water was then filtered through paper (Whatman's No. 1) to remove suspended particles. The water was removed by freeze-drying and the marker was re-dissolved in acetone.

The marker was evaporated onto 400 conditioned cowpeas so that each cowpea was coated with the equivalent of 4.5 eggs. In addition 400 control (unmarked) cowpeas were prepared using 250 ml of acetone only.

10 marked cowpeas were placed in each of quadrants one and three of twenty choice chambers. 10 control cowpeas were placed in each of quadrants two and four of the same twenty choice chambers. The choice chambers were placed in the CTH room overnight to allow the temperature of the cowpeas to stabilise. The following morning a newly emerged and mated Campinas pair was placed in each choice chamber. The beetles were left for 24 hours before they were removed, the cowpeas were then left in the CTH room for a week before the eggs were counted and the number which had hatched noted.

Marker concentration

About 800 beetles were placed on 1200 clean glass beads for 48 hours. The beetles were removed and the glass beads were soaked in 500 ml of petroleum ether for 5 minutes (petroleum ether was used since it was as effective as acetone and, on this occasion, more convenient). The petroleum ether was then decanted and the beads were rinsed in a further 100 ml of petroleum ether. This was added to the original liquid to produce 600 ml of marker.

300 ml of this marker was evaporated onto 400 conditioned cowpeas to produce individual cowpeas coated with the equivalent of about 4 eggs per cowpea. 150 ml of fresh petroleum ether was added to 150 ml of the remaining marker and this was evaporated onto another 400 conditioned cowpeas to produce individual cowpeas marked with the equivalent of about 2 eggs per cowpea. This was repeated with 75 ml and 34 ml of the remaining marker to produce 400 cowpeas marked with the equivalent of about 1 egg per cowpea and 400 cowpeas marked with the equivalent of about half an egg per cowpea. In addition four lots of 400 control cowpeas were prepared using 200 ml of petroleum ether alone for each 400 cowpeas.

10 marked cowpeas coated with marker of a particular strength were placed in each of quadrants 1 and 3 of twenty choice chambers. 10 control cowpeas were placed in each of quadrants 2 and 4 of the same choice chambers. Thus there were 80 choice chambers. 20 of these each offered the choice between 20 control cowpeas and 20 cowpeas marked with the equivalent of about 4 eggs per cowpea, 20 more offered a similar choice between control cowpeas and cowpeas marked with the equivalent of about 2 eggs per cowpea. Of the remaining 40 choice chambers, 20 offered a similar choice with cowpeas marked with the equivalent of about 1 egg per cowpea and the last 20 offered the choice with cowpeas marked with the equivalent of about half an egg per cowpea.

The choice chambers were left in the CTH room overnight so that the temperature of the cowpeas could stabilise. The following morning a newly emerged, mated Campinas pair was placed in each choice chamber for 24 hours. The cowpeas were left for one week before the eggs were counted and egg hatching noted.

Marker decay

Four lots of marker were prepared. These were of equal strength and each was dissolved in 250 ml of petrol ether. When one aliquot of marker was evaporated onto 300 cowpeas each cowpea was coated with the equivalent of about 4 eggs per cowpea.

One aliquot of marker in solvent was evaporated onto 300 conditioned cowpeas. 300 control cowpeas were also prepared at the same time using 250 ml of petroleum ether alone. 10 marked cowpeas were placed in each of quadrants 1 and 3 of fifteen choice chambers and 10 control cowpeas were placed in each of quadrants 2 and 4 of the same choice chambers. These choice chambers were left in the CTH room for the thirty days prior to the start of the experiment.

A second aliquot of marker was evaporated onto 300 conditioned cowpeas fourteen days before the start of the experiment. 300 control cowpeas were also prepared at the same time. These cowpeas were placed in fifteen choice chambers, in the same way as described for the

previous cowpeas, and left in the CTH room for fourteen days prior to the start of the experiment. This procedure was repeated 7 days before the start of the experiment and on the day of the experiment.

This gave four sets of fifteen choice chambers. Fifteen offered the choice between 20 control cowpeas and 20 cowpeas coated with thirty day old marker. Fifteen more offered the choice between control cowpeas and cowpeas coated with fourteen day old marker, fifteen the choice between control cowpeas and cowpeas coated with seven day old marker and the final fifteen offered the choice between control cowpeas and cowpeas coated with 'fresh' marker.

At the start of the experiment a newly emerged, mated Campinas pair was placed in each choice chamber for 24 hours. The beetles were removed and the cowpeas left for one week. The eggs on the cowpeas were then counted and egg hatching was noted.

6.2.3. Results

The percentage of eggs laid on cowpeas coated with marker dissolved using different solvents and that of eggs laid on the respective control cowpeas are shown in Fig. 6.5. In all four cases females laid significantly more ($p < 0.001$) eggs on the control cowpeas than on the marked cowpeas.

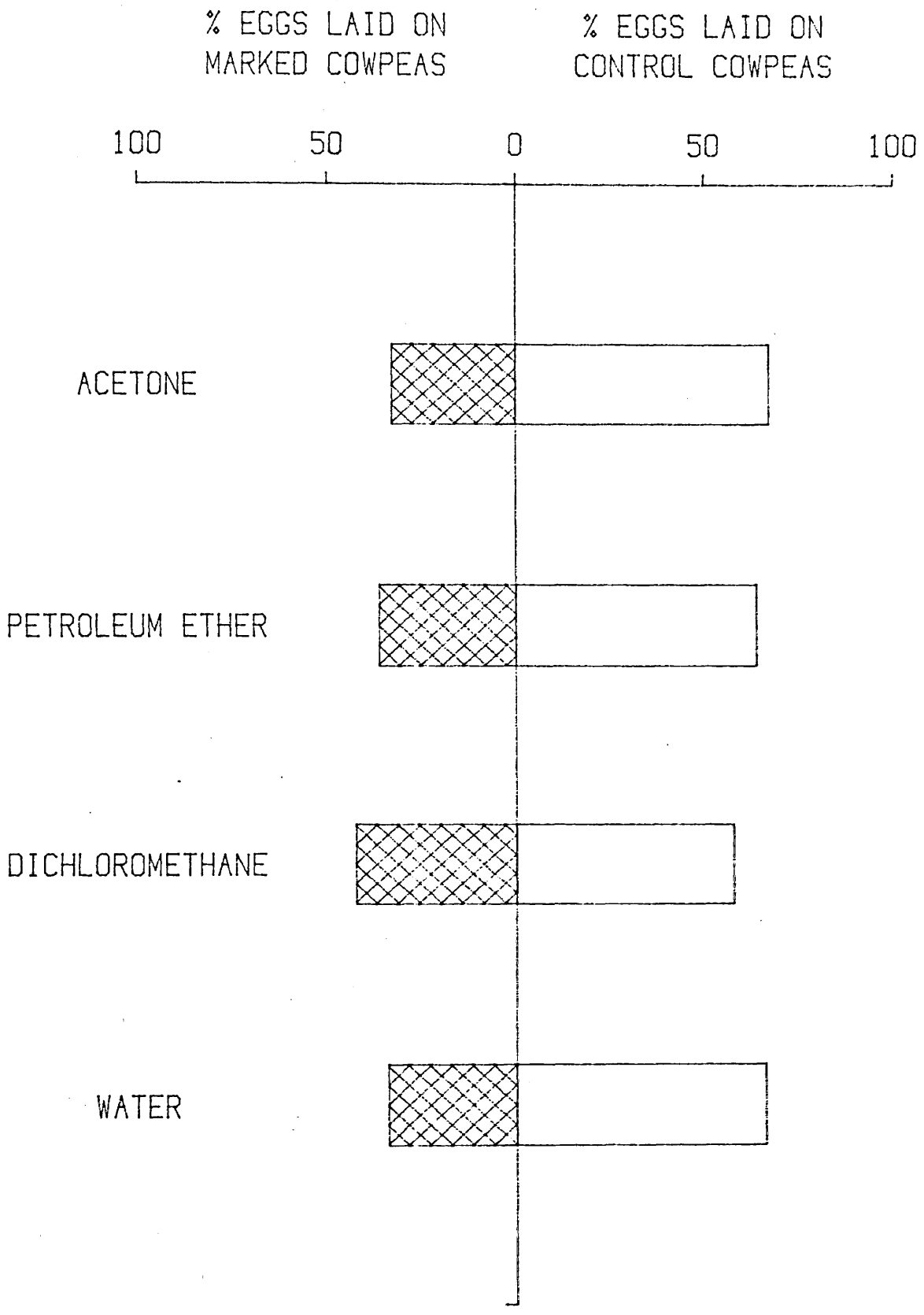


Figure 6.5: Effect of extracting marker with different solvents.

The percentage of eggs (average for twenty replicates) laid over 24 hours on a choice between 20 marked and 20 unmarked (control) cowpeas by newly emerged and mated Campinas females.

The percentage of eggs laid on the cowpeas which were coated with different strengths of marker and on the respective control cowpeas is shown in Fig. 6.6. There were significantly more ($p < 0.05$) eggs laid on the control cowpeas than on the cowpeas coated with marker equivalent to about two or four eggs per cowpea. The difference in the numbers of eggs laid on control cowpeas and cowpeas coated with the equivalent of one or half an egg per cowpea was not significant.

The percentage of eggs laid on cowpeas coated with egg marker at various times before the start of the experiment and that of eggs laid on the respective control cowpeas are shown in Fig. 6.7.

There were significantly more eggs laid on the control cowpeas than on the marked cowpeas when the cowpeas had been coated with marker 30 days before the start of the experiment ($p < 0.05$), 7 days before the start of the experiment ($p < 0.01$) and on the day of the experiment (fresh marker) ($p < 0.05$). There was no significant difference between the number of eggs laid on the marked and control cowpeas when the cowpeas were marked 14 days before the start of the experiment.

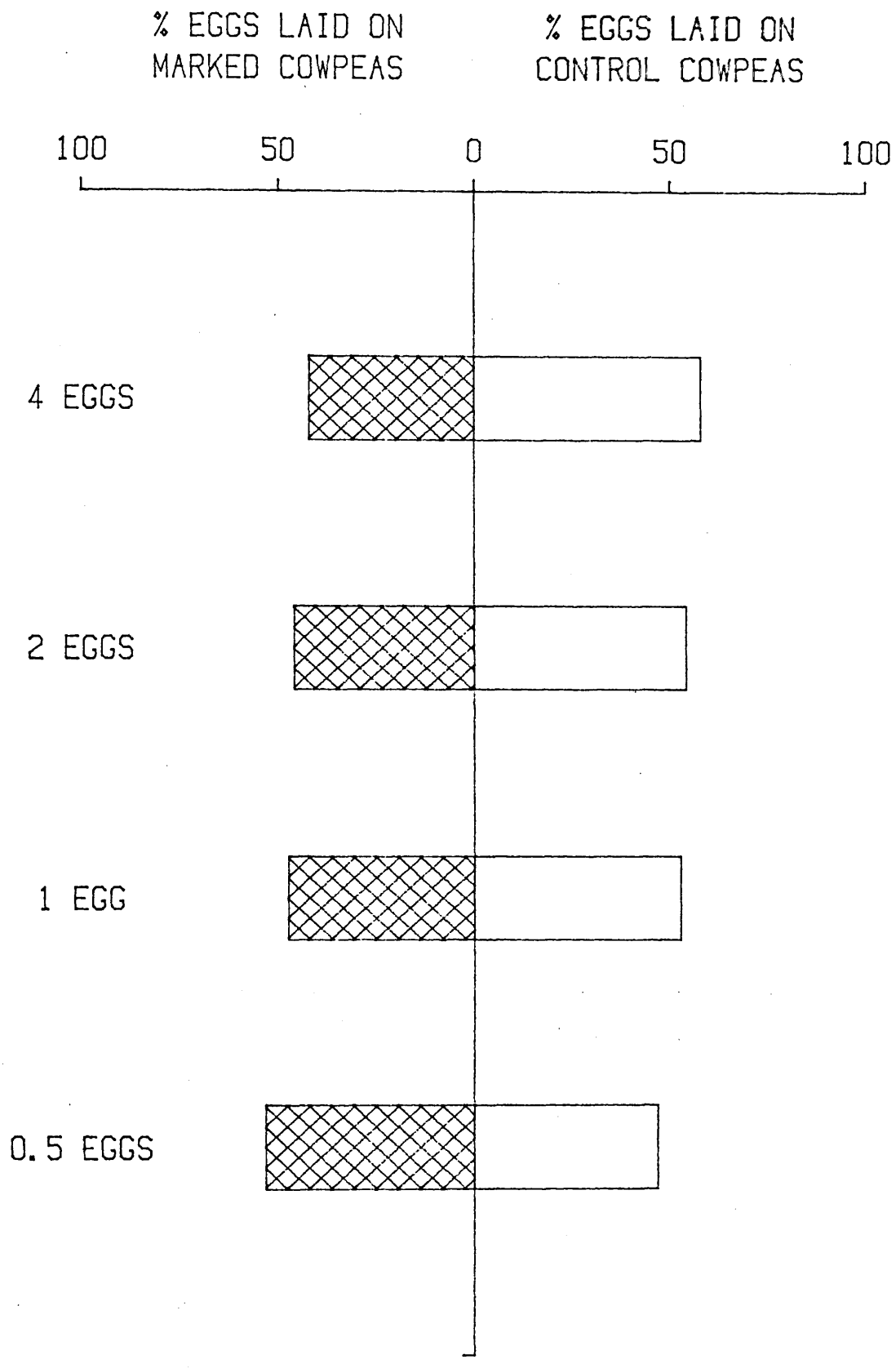


Figure 6.6: Different strengths of marker.

The percentage of eggs (average for twenty replicates) laid over 24 hours on a choice between 20 marked and 20 unmarked (control) cowpeas by newly emerged and mated Campinas females. The approximate marker strength is given in egg equivalents per marked cowpea.

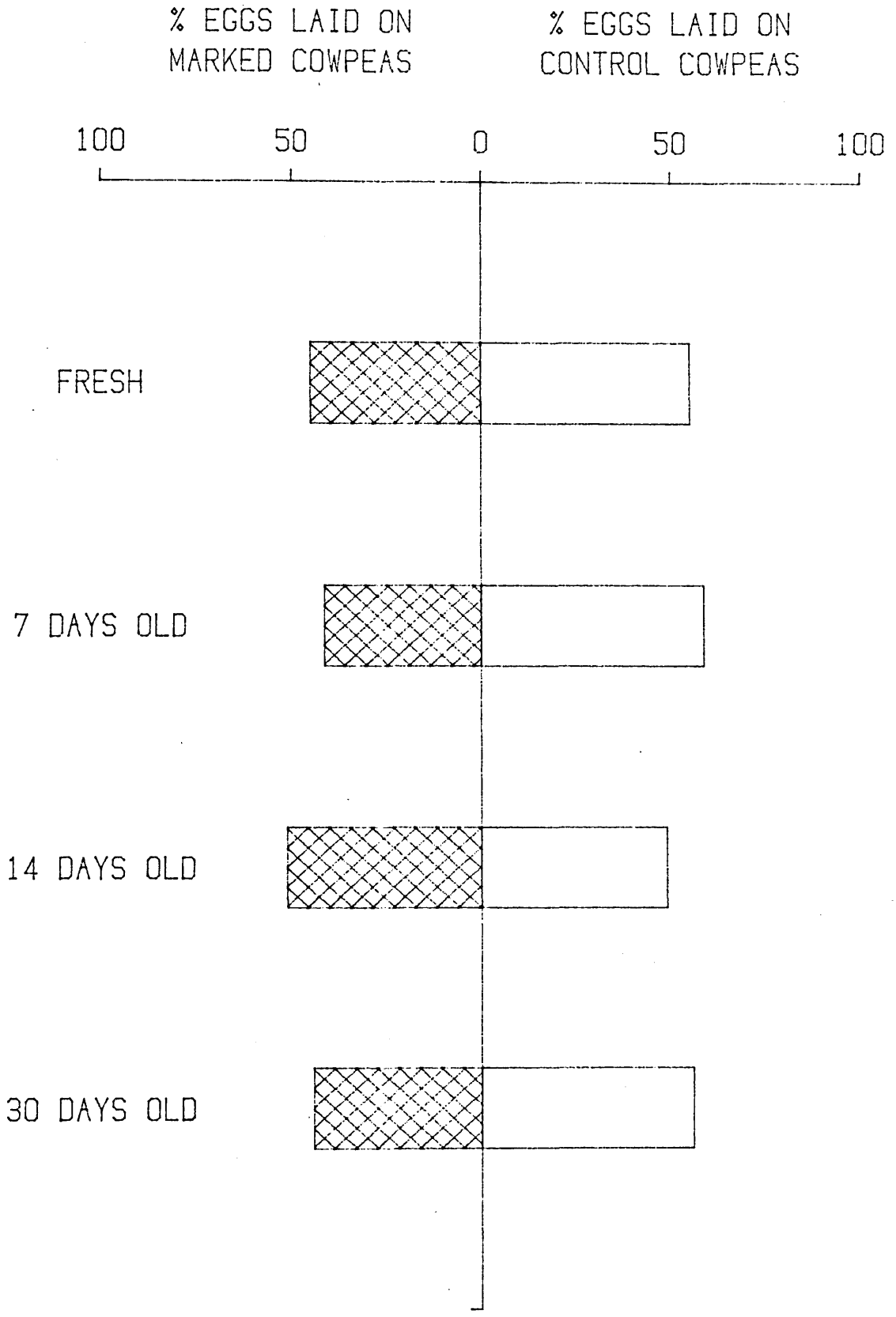


Figure 6.7: Effect of time on marker decay.

The percentage of eggs (average for fifteen replicates) laid over 24 hours on a choice between 20 marked and 20 unmarked (control) cowpeas by newly emerged and mated Campinas females. Times given are for how long the cowpeas were left exposed in the CTH room prior to the start of the experiment.

6.2.4. Discussion

Oshima et al (1973) isolated an oviposition marker deposited by C. chinensis. This marker was fractionated and the different fractions tested using a bioassay; some of the fractions evoked a greater response than others. Whilst this showed something of the nature of the marker it more clearly demonstrated the complexity of the pheromone and the difficulty in identifying the active components.

Because of the difficulties involved in isolating and identifying such pheromones no attempt was made to do this for the egg marker deposited by C. maculatus. Instead preliminary experiments were carried out to give some indication of the nature of the marker. It was hoped that this might be of use in any further and more detailed investigation into the chemical composition of the marker.

The types of solvent used in this study in which the marker was dissolved indicate that the active components are unlikely to be proteins or peptides but it is unusual that the marker could be dissolved in both petroleum ether and water (P. Zagalsky, pers. comm.). It is likely that the marker consists of more than one active component and it may be that one active component is soluble in water and another in petroleum ether. Another reason for this anomaly is that the active component may have been taken up in suspension in the water. Although

the water was filtered to remove suspended matter the filter medium was not fine enough to remove very small particles such as lipid droplets.

The results indicate little of the nature of the marker but did show that petroleum ether is suitable to dissolve the marker and could be used instead of acetone when convenient.

The length of time over which a pheromone remains effective indicates how volatile it is. Ditterick et al (1983) found that an oviposition deterrent produced by the larvae of Ostrinia nubilalis (Lepidoptera: Pyralidae) remained effective for three days. Prokopy (1975) showed that an oviposition deterring pheromone produced by Rhagoletis fausta remained effective for at least nine days. Messina & Renwick (1985a) found that C. maculatus females were deterred from laying eggs on cowpeas with eggs already attached even though the eggs already present were fourteen days old.

In the present study, cowpeas which had been marked thirty days before the start of the experiment deterred the egg laying of C. maculatus but as many eggs were laid on cowpeas which had been coated with marker 14 days before the start of the experiment as were laid on the control cowpeas. This may be due to the abnormal behaviour of one or two of the ovipositing females in the '14 day' group so that whilst the general trend was to avoid marked cowpeas there was no significant difference.

That the marker proved effective after 7 and after 30 days indicates that not all the components are highly volatile and that the pheromone is probably detected by the beetles on contact. Since the proposed function of the egg marker is to reduce the competition between larvae and since competition can occur between larvae of different stages (Bellows, 1982b), a marker which did not evaporate quickly would be best because it would have a lasting effect.

A second advantage of the pheromone's low volatility is that the effect of the marker is discrete - it limits its effect to one cowpea - this is supported by the experiment, previously described, in which the marked and control cowpeas were mixed together.

Assuming that the amount of marker on a cowpea increased as the number of eggs laid on it increased, and this is not proven, one might expect a female to be able to recognise as infested a cowpea with a heavy egg load more easily than one with few eggs. This would have the advantage of enabling females to choose between cowpeas with different egg loads and females in the field would often be faced with such a choice.

In the present study as the strength of the marker was increased so the ability of females to discriminate between marked and control cowpeas was increased, i.e. the proportion of eggs laid on the marked cowpeas decreased. Females in chambers offering the choice

between control cowpeas and cowpeas coated with marker equivalent to 1 egg per cowpea or less did not lay significantly less eggs on the marked cowpeas.

In the previous section of this chapter it was shown that females can distinguish more readily between marked and control cowpeas if these are mixed together. If the cowpeas coated with marker equivalent to one or half an egg per cowpea were mixed in the same way with unmarked cowpeas, females may be able to distinguish between the two.

Although females appear to detect marked cowpeas more readily if the strength of the marker is increased, this experiment only presented females with the choice between marked and unmarked cowpeas. It would be valuable to test the ability of females to discriminate between cowpeas with different egg loads. This was carried out and is discussed in the next chapter.

6.3. Source of the marker and strain comparisons

6.3.1. Introduction

Throughout this thesis the term egg marker has been used to describe the substance or substances deposited by adult beetles which deters egg laying by females on cowpeas marked with it. The term implies that the substance actually indicates the presence of an egg and is specifically associated with eggs. This is not proven.

Yoshida (1961) describes how C. maculatus and C. chinensis adults of both sexes 'conditioned' (marked) beans by walking over them. Szentesi (1981) described how A. obtectus adults deposited substances on beans during various activities. The substances deposited by these species were found to deter oviposition but in neither of these studies was it implied that oviposition was necessary for the production of the oviposition deterrent.

Experiments were carried out to investigate the origin of the oviposition deterrent. Of particular interest was whether both males and females produced a marker and if so which sex produced the most. Szentesi (1981) held that A. obtectus males produced more marker than females whilst Oshima et al (1973) stated that the opposite was the case for C. chinensis.

Experiments were also devised in an attempt to demonstrate the role of oviposition in marker production since oviposition might facilitate the production of marker by females. This would seem a logical occurrence for the production of an oviposition deterrent since its function is to prevent egg laying on cowpeas with eggs already attached.

In previous experiments females of the IITA strain, unlike those of the other two strains, did not suppress their egg laying when placed on only two cowpeas for six days. One possible reason for this was presented in

Chapter 3 but another explanation may be that females of the IITA strain do not recognise or do not produce egg marker. In this case IITA females might not decrease their egg laying as the egg load on a cowpea builds up because they would not detect the egg marker.

To test whether this was possible females of the different strains were presented with cowpeas coated with markers from their own and other strains. This would show if the females produced a marker and if they responded to it. It would also show if females of one strain would respond to marker produced by another strain.

6.3.2. Materials and methods

Female marker

400 females were placed on 500 clean glass beads for twenty-four hours. The females were then removed and 250 ml of acetone was poured onto the beads for thirty minutes. This was decanted and the beads rinsed in 50 ml of fresh acetone which was added to the original 250 ml. This gave 300 ml of female marker.

The marker was evaporated onto 400 cowpeas to give cowpeas marked with an equivalent of about 2.4 eggs per cowpea. 400 control cowpeas were also prepared using 250 ml of acetone alone. Twenty choice chambers were prepared by placing 10 marked cowpeas in each of quadrants 1 and 3, and 10 control cowpeas in each of

quadrants 2 and 4. These were left in the CTH room overnight.

A newly emerged mated Campinas pair was placed in each choice chamber. These were removed twenty-four hours later and the cowpeas were left for a week. The eggs on the cowpeas were counted and hatching was noted.

Female marker with no eggs laid

200 Campinas females were placed onto 700 clean glass beads in a crystallizing dish. To prevent the females from laying eggs the beads were agitated every twenty seconds for half a second by rotating the crystallizing dish mechanically. The beetles were removed after 24 hours and the beads were checked for eggs. Eggs, or traces of eggs, were found on 62 beads which were removed.

250 ml of acetone was poured onto the remaining beads for 10 minutes. This was decanted and the beads were rinsed in a further 50 ml of fresh acetone which was added to the 250 ml to give 300 ml of female marker.

The marker was evaporated onto 300 cowpeas and 300 control cowpeas were also prepared using 250 ml of acetone alone. Fifteen choice chambers were prepared by placing 10 marked cowpeas in each of quadrants 1 and 3, and 10 control cowpeas in each of quadrants 2 and 4. The choice chambers were left in the CTH room overnight for

the temperature to stabilise.

The following morning a newly emerged mated Campinas pair was placed in each choice chamber. These were removed twenty-four hours later and the cowpeas were left for a week. The eggs on the cowpeas were counted and hatching was noted.

Male marker

400 males were placed on 500 clean glass beads for twenty-four hours. The males were then removed and every bead was checked for eggs, since the odd female would inevitably be included. 15 eggs, or traces of eggs, were found and the beads they were on removed. 250 ml of acetone was then poured onto the beads (in a clean crystallizing dish) for thirty minutes. This was decanted and the beads rinsed in 50 ml of fresh acetone which was added to the original 250 ml. This gave 300 ml of male marker.

For practical reasons the marker was not evaporated onto cowpeas until four months later and for several weeks of this the marker was not refrigerated. The marker was then evaporated onto 400 cowpeas and 400 control cowpeas were also prepared using 250 ml of acetone alone. Twenty choice chambers were prepared by placing 10 marked cowpeas in each of quadrants 1 and 3, and 10 control cowpeas in each of quadrants 2 and 4.

A newly emerged mated Campinas pair was placed in each choice chamber. These were removed twenty-four hours later and the cowpeas were left for a week. The eggs on the cowpeas were counted and hatching was noted.

Strain comparison

Five hundred clean glass beads were placed in each of three crystallizing dishes. Approximately 400 unsexed beetles of the Campinas strain were placed on one lot of cowpeas for twenty four hours. 400 unsexed beetles of the IITA strain were placed on a second lot of cowpeas and 400 unsexed beetles of the Yemen strain were placed on the third. These were collected at the same time as the Campinas beetles and also left on the cowpeas for twenty-four hours. After twenty-four hours the beetles on each lot of glass beads were removed and discarded.

250 ml of acetone was poured onto each set of beads for twenty-five minutes and then each was decanted into a separate glass container. Each lot of beads was rinsed in a further 50 ml of acetone and these three 50 mls were added to their respective 250 mls. These three markers were Campinas marker, IITA marker and Yemen marker. The exact strength of each, in egg equivalents, was not equal but as the purpose of the experiment was only to demonstrate the response of one strain to marker produced by another strain and not the degree of response this was not important (the strengths, where known, are given in

Table 6.3: Effectiveness of the marker of the three different strains at deterring the oviposition of females from each strain.

Strain of females on cowpeas	Strain of beetles producing marker		
	Campinas	IITA	Yemen
Campinas	33.74 / ?	25.31 / ?	17.60 / ?
IITA	32.83 / 2.85	24.14 / 3.8	18.12 / 4.5
Yemen	19.42 / 2.6	14.14 / 3.15	6.74 / 5.6

The first figure in each case is the percentage of eggs laid on the marked cowpeas in the choice chamber and the second figure is the strength of the marker, in egg equivalents, on the marked cowpeas.
In all cases there were significantly more eggs laid on the control cowpeas than on the marked cowpeas.

Table 6.3).

The markers were each evaporated onto 400 conditioned cowpeas in the usual way. Three lots of 400 control cowpeas were also prepared using 250 ml of acetone alone. 10 cowpeas, coated with marker from a particular strain, were placed in each of quadrants 1 and 3 of twenty choice chambers and 10 control cowpeas were placed in each of quadrants 2 and 4 of the same choice chambers. This gave a total of sixty choice chambers; twenty offered the choice between cowpeas coated with Campinas marker and control cowpeas, twenty offered the choice between cowpeas coated with IITA marker and control cowpeas and twenty offered the choice between cowpeas coated with Yemen marker and control cowpeas. These choice chambers were then transferred to the CTH room and left overnight.

A newly emerged Campinas pair was placed in each choice chamber and removed twenty-four hours later. The cowpeas were left for a week and the eggs counted, egg hatching was also noted.

This experiment was repeated a second and a third time presenting the same choices to the other two strains of females. Thus, IITA females were offered choices between cowpeas coated with the marker of a particular strain and control cowpeas; Yemen females were also offered similar choices.

6.3.3. Results

The percentage of eggs laid by females on the cowpeas coated with a marker deposited by egg laying females is shown in Fig. 6.8. Significantly more eggs were laid on the control cowpeas than on the marked cowpeas ($p < 0.001$). The percentage of eggs laid by females on cowpeas coated with a marker deposited by other females which were prevented from laying eggs is also shown in Fig. 6.8. Significantly more eggs were laid on the control cowpeas than on the marked cowpeas ($p < 0.01$). Fig. 6.8 also shows the percentage of eggs laid by females on cowpeas coated with a marker deposited by males. Significantly more eggs were deposited on the control cowpeas than on the marked cowpeas ($p < 0.05$).

Females avoided ovipositing on cowpeas coated with the marker of any of the three strains. When presented with the choice between cowpeas coated with the marker of any of the three strains and unmarked cowpeas females laid significantly ($p < 0.05$) less eggs on the marked cowpeas. The percentages of eggs laid on the marked cowpeas and the strength of the egg markers (in egg equivalents) are shown in Table 6.3.

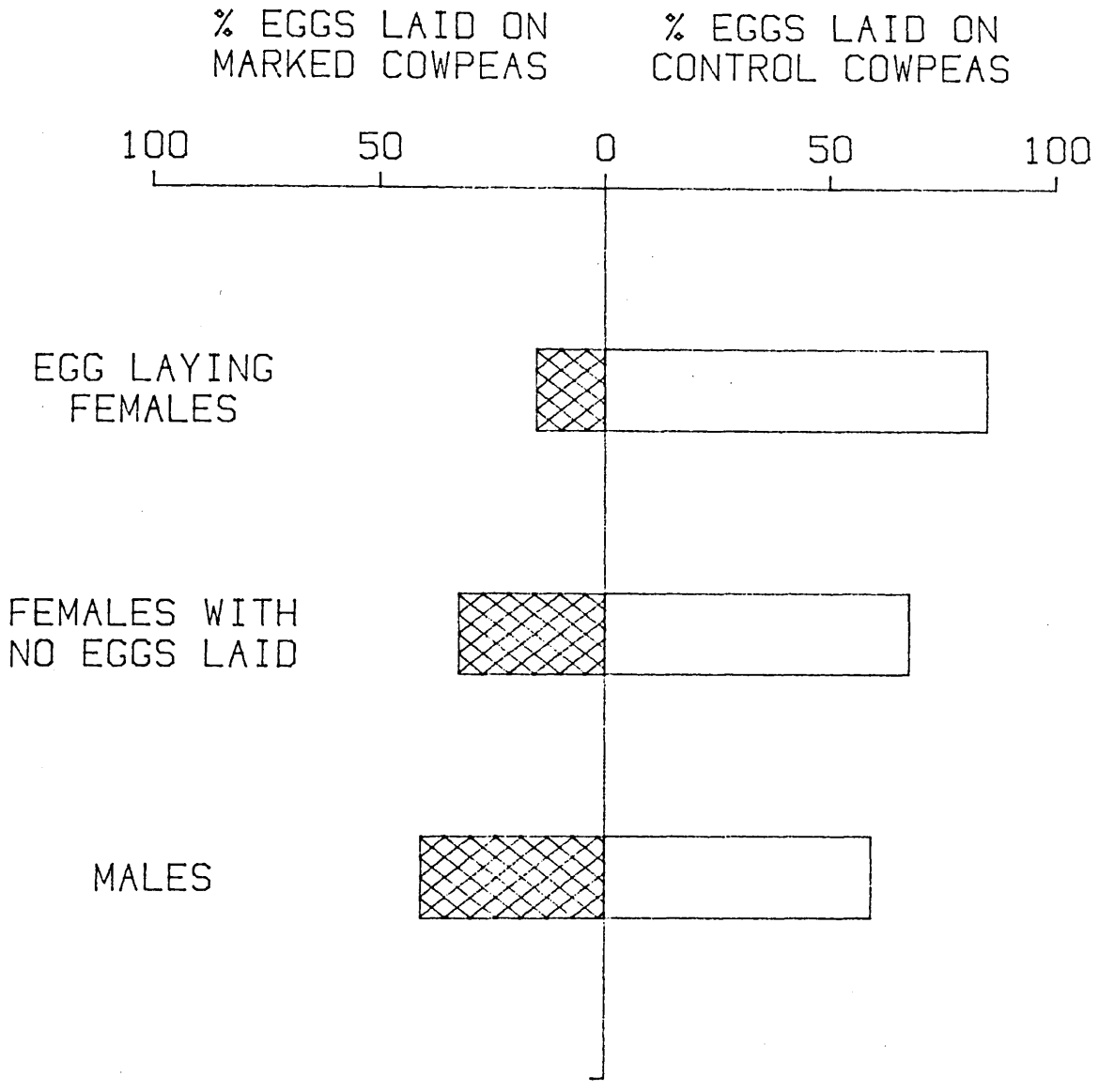


Figure 6.8: Marker from different sexes.

The percentage of eggs (average for twenty replicates for males and egg laying females and average of fifteen replicates for females laying no eggs) laid over 24 hours on a choice between 20 marked and 20 unmarked (control) cowpeas by newly emerged and mated Campinas females. The different groups which deposited the marker are shown - the females with no eggs laid were mated but were artificially prevented from laying eggs by intermittant agitation of the glass beads they were placed on.

6.3.4. Discussion

Oviposition deterring pheromones are usually associated with eggs deposited on a substrate (Prokopy, Greany & Chambers, 1977; Behan & Schoonhoven, 1978; Zimmermann, 1979) but substances deposited at other times may also deter females from ovipositing. Price (1970) found that females of several genera of Hymenoptera, when searching for egg laying sites, avoided areas which had already been searched and that a 'trail odour' was responsible. Yoshida (1961) demonstrated that adults of C. chinensis and C. maculatus deposited a substance when walking over beans which deterred females from ovipositing; this substance could be removed using ether.

Oshima et al (1973) working on C. chinensis and Szentesi (1981) working with A. obtectus found that adult males deposited substances whilst crawling over beans which deterred subsequent oviposition. Yoshida (1961) found that C. maculatus males could apparently make beans less attractive for oviposition by crawling over them.

C. maculatus females, which were artificially prevented from laying eggs, deposited a substance which acted as an oviposition deterrent. Males also deposited a substance on glass beads which acted as an oviposition deterrent. In previous experiments (described in section 6.1) mixed-sex groups of beetles have been shown to deposit an oviposition deterrent and in this series of experiments ovipositing females were shown to deposit an

oviposition deterrent.

The experiments presented in this study have demonstrated that the oviposition deterring pheromone of C. maculatus comes from several sources and not just those associated with egg production and oviposition. What is less clear is the relative importance of these sources in pheromone production.

It must be mentioned that the term oviposition deterring pheromone is used here in singular form in preference to the plural. Although it is recognised that the oviposition deterring pheromone comes from different sources and that these components may have different structures and properties of their own, in the context of oviposition deterrence they have a single function. To save confusion the singular term is used throughout.

The substance deposited by females which were artificially prevented from ovipositing was more effective than that deposited by males (Fig. 6.8). This may be because they deposited more of the same substance or because they deposited different, and more effective, substances than males. The females were constantly disturbed as the glass beads they were on were rotated - this may result in less material being deposited than normal or, alternatively, the deposition of some 'stress-induced' substance which also acts as an oviposition deterrent.

Oshima et al (1973) found that the number of different kinds of substances deposited by C. chinensis females was greater than that of substances deposited by males. They also found that the substances deposited by females 'released about 4 times more activity' than that of males (although in their experiment females were allowed to oviposit). In this respect C. maculatus more closely resembles C. chinensis than A. obtectus since Szentesi (1981) found that the male marker of A. obtectus was more effective than that of the female.

It would be rare to find large single-sex groups of adults on cowpeas and the mixed-sex groups usually to be found would be engaging in the normal activities of mating and egg laying. Markers produced by groups of beetles of mixed-sex are more effective than those deposited by males or by mated females which were artificially prevented from laying eggs. It may be interesting to see whether virgin females also produce an oviposition deterring substance though they would be difficult to collect in sufficient numbers.

Amongst groups of similar size, so far investigated, the most effective marker was deposited by an all female group; the females were of mixed ages, presumably most had mated, and they were allowed to lay eggs. The results of this experiment suggest that the strength of the oviposition deterring pheromone is somewhat independent of the number of eggs laid. The strength of

the marker produced by the females was equivalent to 2.4 eggs per cowpea whilst that of a marker produced by a mixed-sex group, in a comparable experiment, was 4.4 eggs per cowpea - yet the marker produced by the female-only group appeared to have a greater deterrent effect (15.2% of eggs on the 'female-only' marked cowpeas compared with 32.6% of eggs on the cowpeas coated with the 'mixed-sex' marker).

Thus it appears that females are a more important source of marker than males and that, whilst more marker is deposited when females are allowed to lay eggs, the effectiveness of the marker is somewhat independent of the number of eggs laid during the deposition of the marker. Until the marker is identified and the amount can be reliably measured, the validity of "egg equivalents" should not be over emphasised.

In view of the results of Oshima *et al* (1973) for *C. chinensis* it seems highly probable that the oviposition deterring pheromone of *C. maculatus* is composed of several different substances. It is also quite conceivable that the source of the pheromone might be two-fold; an adult marker deposited as beetles walk over a surface (the 'conditioning factor' described by Yoshida, 1961) and an actual egg-marker produced with the eggs (though the quantity of this second component might not be related to the number of eggs laid).

It is not possible to distinguish between different elements of the pheromone using the techniques described here and so further solutions to the problem concerning the source of the marker await isolation and identification of the various active components of this pheromone.

The term "oviposition deterring pheromone", whilst unwieldy, more accurately describes the nature of the pheromone than "egg marker". Despite the fact that the pheromone is not necessarily associated with eggs it was much more evident when oviposition occurred and this would be the usual circumstances of its greatest production anyway.

As a result of the experiments investigating the suppression of egg laying by females of the Campinas and Yemen strains on a reduced number of cowpeas, and the lack of suppression exhibited by females of the IITA strain, the ability of females of one strain to recognise the markers deposited by beetles of the other two strains was investigated. It was thought that the IITA strain might not produce an oviposition deterring pheromone or that the females of that strain might not recognise such a pheromone, partly explaining their lack of suppression on a limited number of cowpeas.

The markers produced by beetles of any of the three strains were recognised by the females of the other two strains and of their own strain. The lack of suppression of egg laying shown by IITA females was not due to their inability to produce or recognise their own egg marker.

Unfortunately the design of the experiment does not allow an accurate assessment of which strain's marker caused the greatest degree of deterrence since the strengths were not known in all cases. It did appear, however, that Yemen females more strongly avoided marked cowpeas than females of the other two strains and that the Yemen marker was more effective than those of the other two strains. These observations warrant further investigation under more rigorous experimental conditions but it does seem logical that Yemen females should more strongly avoid marked cowpeas than females of the other two strains. This is because only a few Yemen adults usually emerge from any one cowpea (Dick & Credland, 1984) and so the benefit to a Yemen female of avoiding cowpeas already with eggs on, even at low egg densities, is potentially greater than for females of the other two strains.

Chapter 7

PHYSICAL FACTORS IN OVIPOSITION STRATEGY

7.1. Introduction

In experiments already described which investigated the factors influencing a female's choice of oviposition site, only the oviposition deterring pheromone has been considered. Every reasonable effort was made to reduce the variation in the physical characteristics of the oviposition substrate; cowpeas were graded for size and those with damaged seedcoats removed.

Under normal circumstances the cowpeas or other seeds on which a female laid its eggs would not be physically identical and there are many references to the way in which the physical characteristics of the oviposition substrate affect the oviposition strategy of C. maculatus and other bruchids.

The size of the seed has been shown to affect the choice made by C. maculatus. Females have been described as preferring larger seeds to smaller ones (of the same species or variety) (Mitchell, 1975; Nwanze & Horber, 1975; Nwanze et al, 1975). This relationship between seed size and ovipositional preference has also been described for C. chinensis by Jakhamola & Singh (1971) although this is in apparent contradiction with the results of Avidov, Berlinger & Applebaum (1965b). Avidov

et al (1965b) found that C. chinensis females tended to avoid larger steel balls when offered a choice between various sizes.

Another important physical factor in the choice of an oviposition site is the texture of the seedcoat, particularly on cowpeas (whose seedcoat texture can vary considerably from one to another). C. maculatus females tend to avoid ovipositing on cowpeas with rough seedcoats (Larson, 1927; Booker, 1967; Nwanze & Horber, 1975; Nwanze et al, 1975); this also applies to C. chinensis (Srivastava & Bhatia, 1959; Totia & Singh, 1966). C. maculatus and C. chinensis females also prefer seeds which are 'well filled' (i.e. whose seedcoats are not loose) (Larson, 1927; Srivastava & Bhatia, 1959; Teotia & Singh, 1966).

By using glass beads of different sizes it was possible to perform a similar experiment to that of Avidov et al (1965b) on C. maculatus. No attempt was made to investigate the effect of seedcoat texture because of the difficulty in quantifying its roughness.

Experiments similar to those of Mitchell (1975), who used mung beans, Phaseolus aureus., were carried out to see if the the size of cowpeas affected the choice of an oviposition site by C. maculatus.

Many experiments have been described in the previous chapter in which the effect of the oviposition deterring pheromone was investigated but a major factor in a female's choice of oviposition site is the physical presence of eggs on the surface of cowpeas (along with any marker deposited at oviposition). An experiment was designed to see if females distinguished between cowpeas with different egg loads.

7.2. Materials and methods

7.2.1. Size of oviposition substrate

Glass beads were used to examine the effect of different sizes of oviposition substrate on the ovipositional behaviour of female beetles. In Chapter 3 it was demonstrated that glass beads coated in a '10 minute' host-plant extract are a suitable artificial substitute for oviposition experiments and so glass beads coated in a similar way were used here. By coating glass beads of different sizes together in the rotary evaporator with the same host-plant extract it was possible to ensure that they all had they same amount of host-plant extract per unit of surface area.

450 ml of acetone was poured onto 1000 cowpeas and left for 10 minutes; the cowpeas had been carefully checked to remove all those with damaged seedcoats. The acetone was decanted and the cowpeas rinsed in a further 50 ml of acetone. The 50 ml was added to the original

450 ml to give 500 ml of host-plant extract.

400 large glass beads (diameter 8.5-9.5 mm, surface area approximately 254 mm^2 per bead) and 400 medium-sized glass beads (diameter 6.5-7.5 mm, surface area approximately 154 mm^2 per bead) were placed together in an evaporating flask. The host-plant extract was poured onto them and the acetone evaporated under reduced pressure at 30 °C. This process took about fifteen minutes.

Twenty glass beads of each size, now coated in host-plant extract, were placed in each of twenty Petri dishes and the dishes left overnight in the CTH room. The following morning a newly emerged and mated Campinas pair was placed in each of the Petri dishes. The beetles were left, undisturbed, for twenty-four hours before they were removed. The eggs on the two sizes of glass bead were then counted (since they were laid on glass beads it was not necessary to wait for hatching).

This experiment was repeated using medium-sized (diameter 6.5-7.5 mm) and small glass beads (diameter 3.5-4.5 mm, surface area approximately 50 mm^2 per bead). Since the total surface area of the beads was smaller than in the first experiment only 800 cowpeas were used to prepare the host plant extract. The same procedure was followed as in the previous experiment, offering the choice between twenty medium-sized beads and twenty small beads to each of twenty pairs of Campinas beetles.

Following these two experiments a further one was designed using all three sizes of glass beads (large, medium and small). A host-plant extract was prepared by pouring 250 ml of acetone onto approximately 600 cowpeas. This acetone was decanted after 10 minutes and the cowpeas rinsed in a further 50 ml; this 50 ml was added to the original 250 ml to give 300 ml of host-plant extract. Less extract was prepared on this occasion because fewer glass beads were to be used. The host-plant extract was evaporated onto 600 glass beads (200 of each size) at 30 °C, under reduced pressure, in the rotary evaporator.

Ten glass beads of each size were placed in each of twenty Petri dishes and these were left in the CTH room overnight. The following morning a newly emerged and mated Campinas pair was placed in each of the Petri dishes. The beetles were left, undisturbed, for twenty-four hours before they were removed. The eggs on each size of glass bead were counted on the same day.

7.2.2. Cowpea weight

Four lots of 200 cowpeas were prepared. These were NOT graded for size but they were carefully inspected and any with damaged seedcoats removed. Each lot of cowpeas was placed in a separate crystallizing dish and they were conditioned for forty-eight hours in the CTH room.

Five pairs of mated Campinas beetles were placed on the cowpeas in the first crystallizing dish, 10 pairs in the second and 20 in the third. 200 unsexed Campinas beetles were placed in the fourth. Twenty-four hours later the beetles in each of the crystallizing dishes were removed. Each cowpea was weighed and the eggs laid on it counted. Because egg hatching was not to be measured and because larval activity might have affected the weight of the cowpeas, the eggs were counted and the cowpeas weighed soon after the beetles were removed.

7.2.3. Recognition of cowpeas with different egg loads

Approximately 2000 sieved and conditioned cowpeas were placed in a crystallizing dish and 350 unsexed Campinas beetles added. The beetles were left on the cowpeas overnight and removed the following morning.

That same day each of the cowpeas were inspected (using tweezers to avoid cross-contamination) until 100 each of cowpeas bearing 1, 2 and 3 eggs were collected. Individual cowpeas of these three groups were labelled '1', '2' and '3' respectively using red water-soluble ink. Care was taken to avoid cross-contamination during labelling.

Five cowpeas of each type (i.e. those with 1,2 or 3 eggs) were placed in each of twenty Petri dishes. These were placed in the CTH room overnight. The following morning a newly emerged and mated Campinas pair was

placed in each Petri dish. The beetles were left, undisturbed, for twenty-four hours before they were removed. A week later the additional eggs laid on each type of cowpea were counted.

7.3. Results

7.3.1. Size of oviposition substrate

The percentage of eggs laid on large and medium-sized glass beads is shown in Fig. 7.1. Significantly more eggs were laid on the medium-sized glass beads than on the large ones ($p < 0.001$). The percentage of eggs laid on the small and medium-sized glass beads is shown in Fig. 7.2. Significantly more eggs were laid on the medium-sized glass beads than on the small ones ($p < 0.001$).

The percentage of eggs laid by females on small, medium and large glass beads is shown in Fig. 7.3. The distribution of eggs is significantly different ($p < 0.01$) from that which might be expected under the null hypothesis (i.e. an equal percentage of eggs on each type of glass bead) and clearly the greatest proportion of eggs was laid on the medium-sized glass beads.

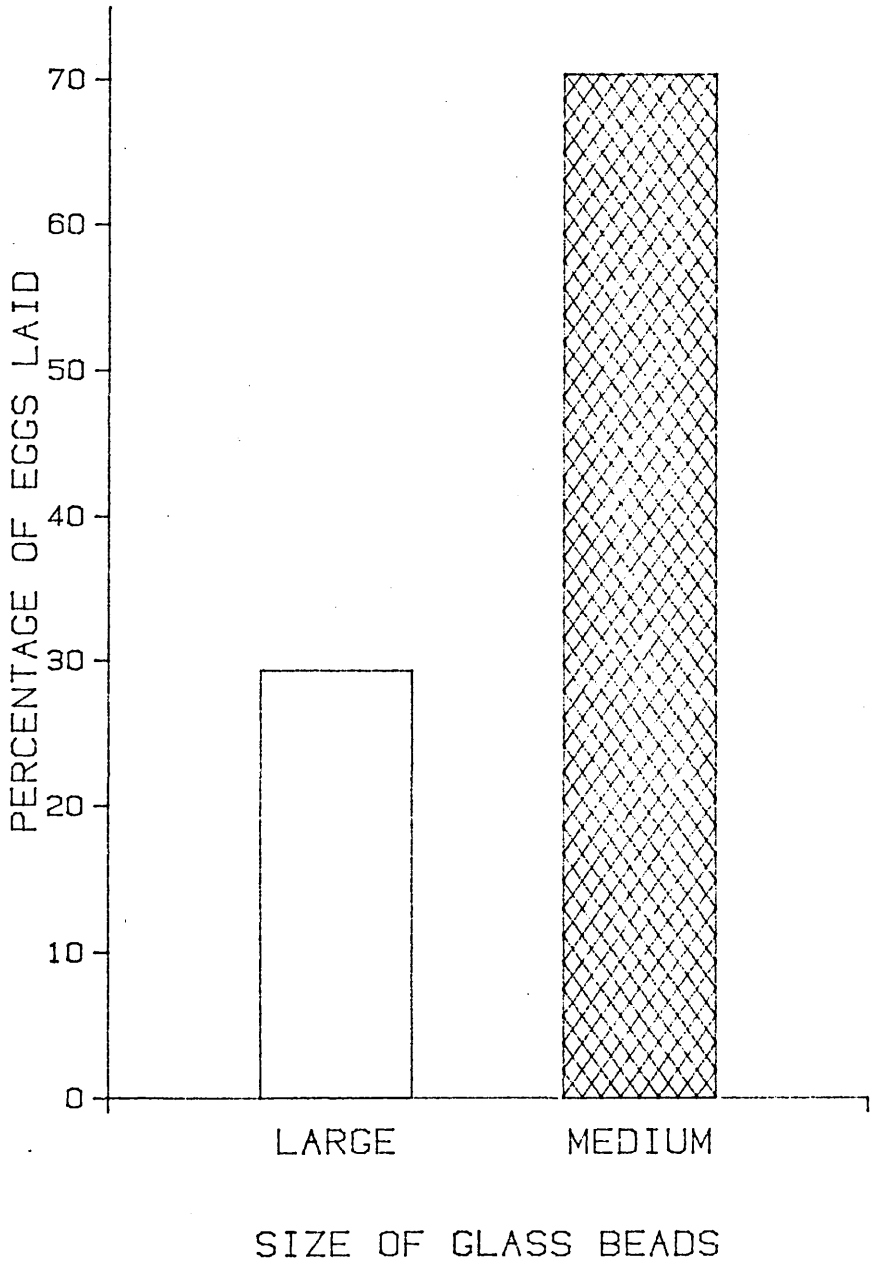


Figure 7.1: Egg laying on large and medium-sized glass beads.

The percentage of eggs (average for twenty replicates) laid over 24 hours by newly emerged and mated Campinas females presented with a choice of twenty large glass beads (diameter 8.5-9.5 mm) and twenty medium-sized glass beads (diameter 6.5-7.5 mm). The glass beads were coated with host-plant extract.

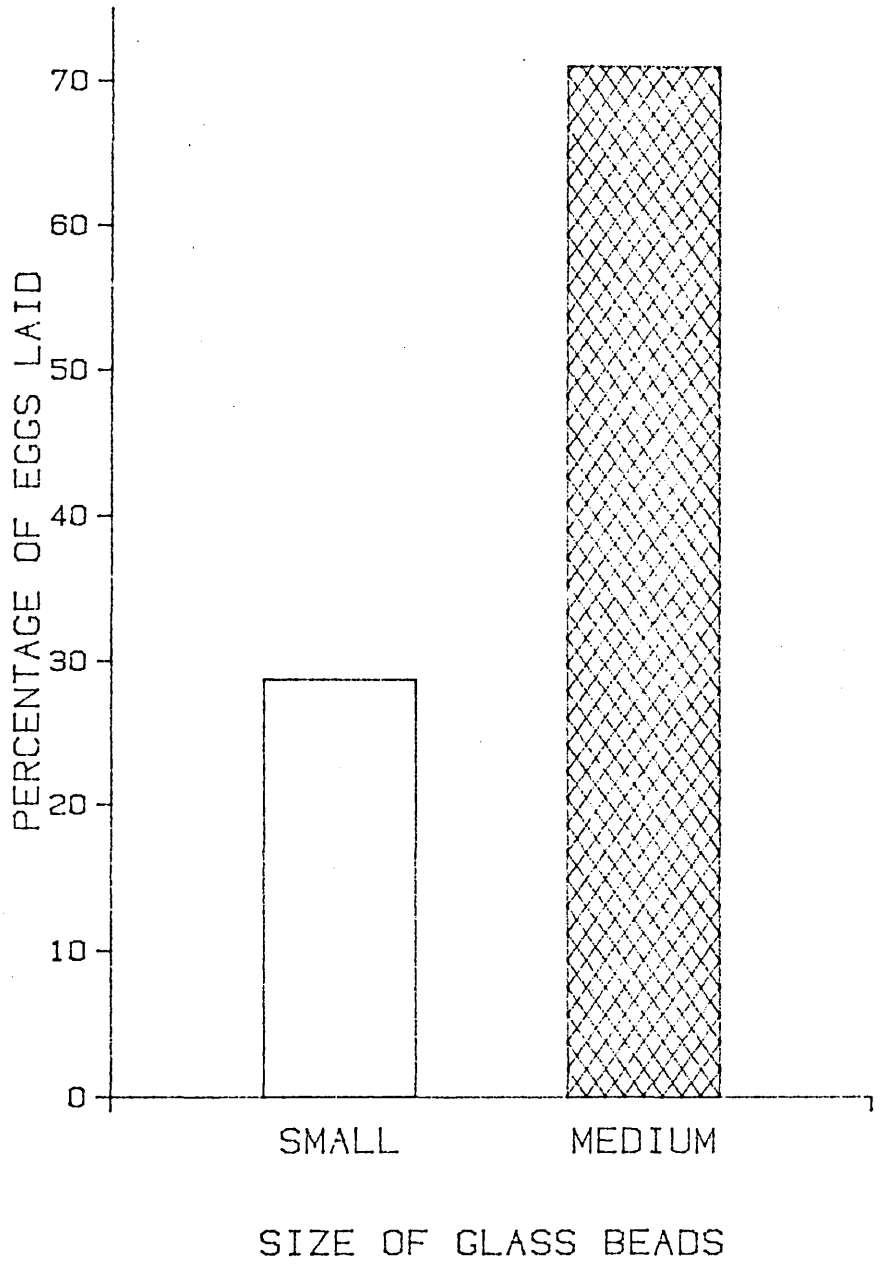


Figure 7.2: Egg laying on small and medium-sized glass beads.

The percentage of eggs (average for twenty replicates) laid over 24 hours by newly emerged and mated Campinas females presented with a choice of twenty small glass beads (diameter 3.5-4.5 mm) and twenty medium-sized glass beads (diameter 6.5-7.5 mm). The glass beads were coated with host-plant extract.

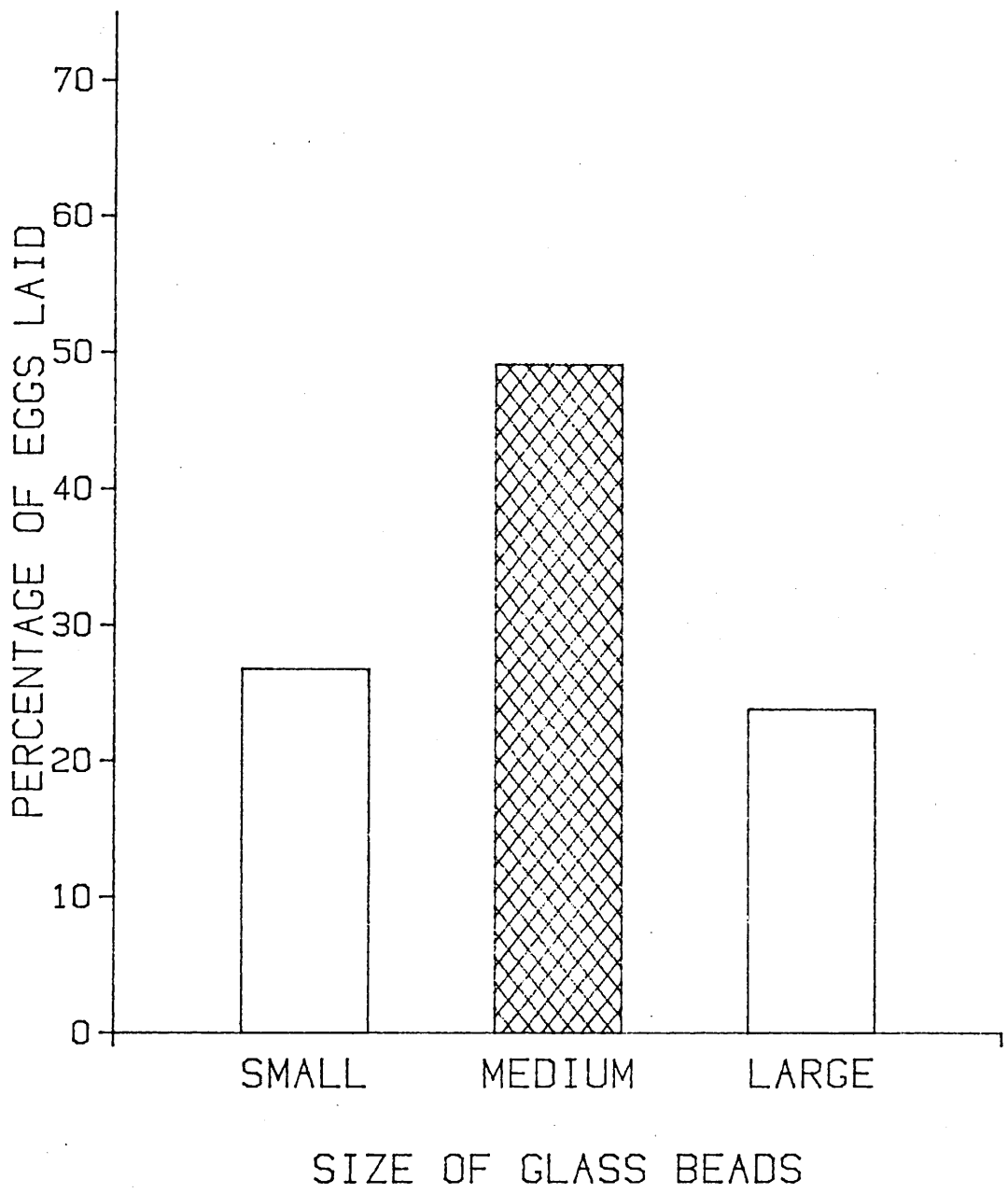


Figure 7.3: Egg laying on small, medium and large glass beads.

The percentage of eggs (average for twenty replicates) laid over 24 hours by newly emerged and mated Campinas females presented with a choice of ten small, ten medium-sized and ten large glass beads (for actual sizes see Figs. 7.1 and 7.2). The glass beads were coated with host-plant extract.

7.3.2. Cowpea weight

The egg densities at the end of the cowpea weight experiment are shown in Table 7.1.

There was no correlation between the weight of a cowpea and the number of eggs which were laid on it for the cowpeas which had 5 or 10 pairs of beetles on them. However, there was a significant correlation between the weight of a cowpea and the number of eggs laid on it for the 20 pairs of beetles ($p < 0.05$) and for the 200 unsexed beetles ($p < 0.001$).

The graph of the number of eggs laid against cowpea weight for the 20 pairs of beetles is shown in Fig. 7.4. A similar graph for the 200 unsexed beetles is shown in Fig. 7.5. Because of the significant correlation in these last two cases a regression analysis was performed on both of them. The regression equations are shown in Table 7.2, both lines are significantly different from the horizontal.

The two regression lines were compared using analysis of covariance. The results of this test is given in Table 7.3. The regression coefficients from the two groups (20 pairs and 200 unsexed beetles) were significantly different. The regression line plotted for the 200 beetles is steeper than that plotted for the 20 pairs.

Table 7.1: Egg densities in the cowpea weight experiment.

Number of beetles	Mean No. of eggs per cowpea	S.E.
10 (5 pairs)	0.442	0.051
20 (10 pairs)	0.980	0.073
40 (20 pairs)	2.559	0.126
200	9.030	0.251

Figure 7.4: Relationship between cowpea weight and oviposition (40 beetles).

Twenty newly emerged and mated Campinas pairs were released onto 200 cowpeas for twenty-four hours. Each cowpea was then weighed and the number of eggs which had been laid on it counted.

The egg density is given in Table 7.1 and the regression equation for the line of best fit is given in Table 7.2. The regression coefficient is significantly different from zero ($p < 0.05$).

\+ - 1 observation
◊ - 2 observations

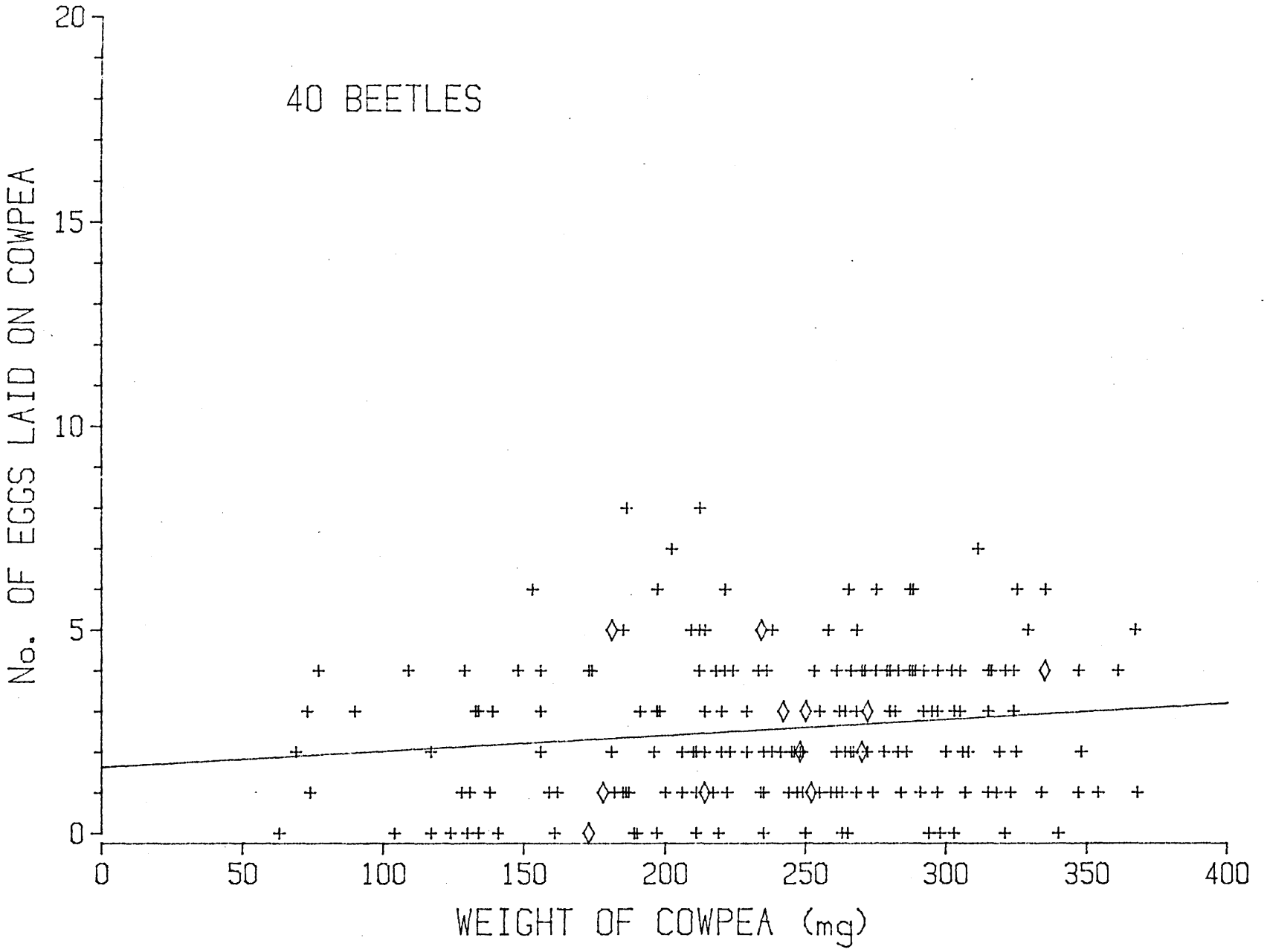

40 BEETLES
No. OF EGGS LAID ON COWPEA
WEIGHT OF COWPEA (mg)
0
5
10
15
20
0
50
100
150
200
250
300
350
400

Figure 7.5: Relationship between cowpea weight and oviposition (200 beetles).

Two hundred unsexed Campinas beetles were released onto 200 cowpeas for twenty-four hours. Each cowpea was then weighed and the number of eggs which had been laid on it counted.

The egg density is given in Table 7.1 and the regression equation for the line of best fit is given in Table 7.2. The regression equation is significantly different from zero ($p < 0.0001$).

\+ - 1 observation
◊ - 2 observations

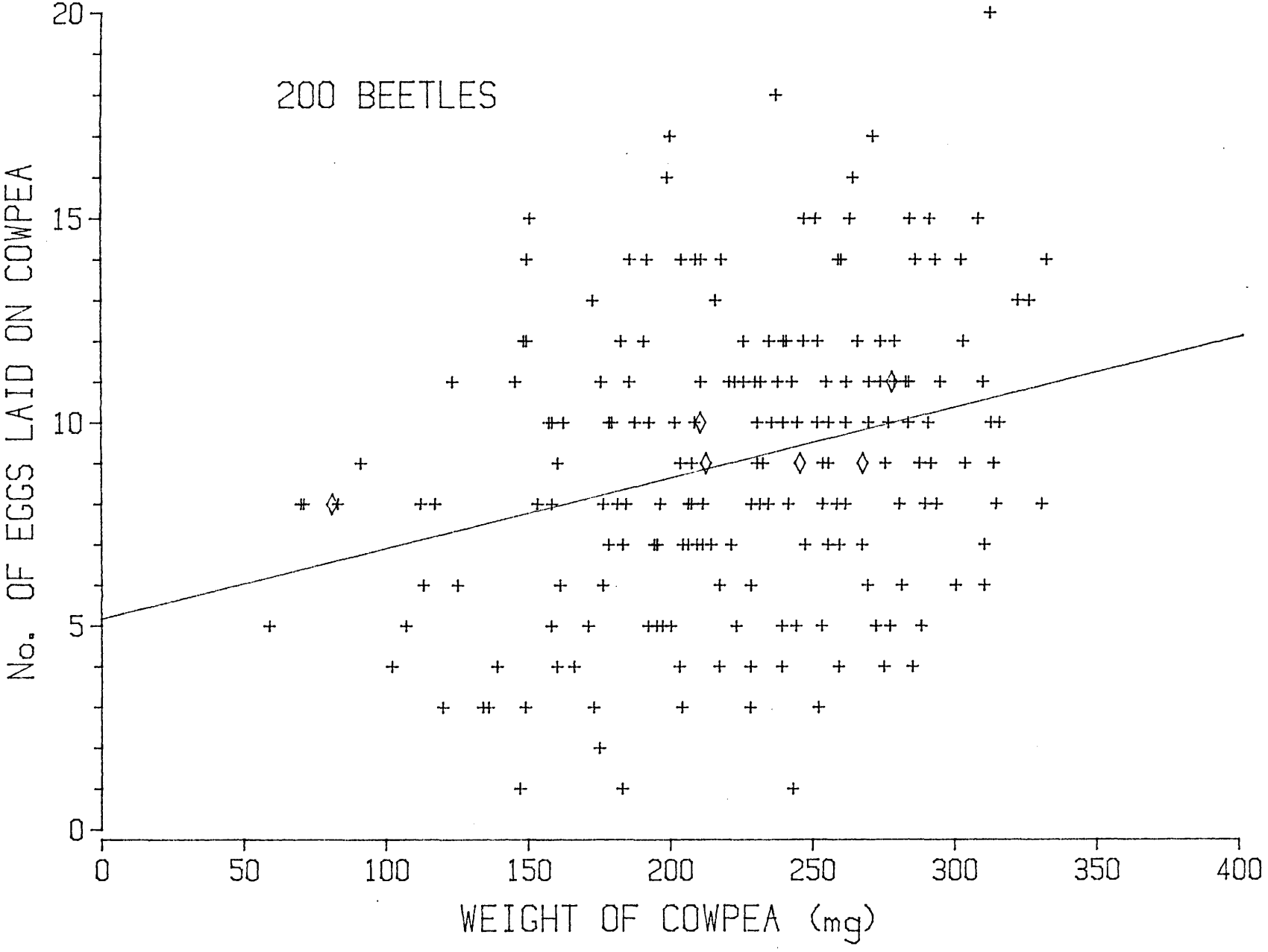

200 BEETLES
No. OF EGGS LAID ON COWPEA
WEIGHT OF COWPEA (mg)
0
5
10
15
20
0
50
100
150
200
250
300
350
400

Table 7.2: Regression equations for number of eggs laid on cowpea weight for 40 and 200 beetles.

Beetle density	Regression equation	
40	No. of eggs = (cowpea wt. x 0.004) - 1.626	*
200	No. of eggs = (cowpea wt. x 0.017) - 5.166	***

Table 7.3: Analysis of covariance for the number of eggs laid by 40 beetles and by 200 beetles on cowpea weight.

Source of variation	DF	SS	MS	F	
Homogeneity of regression coefficients					
Treatments	1	69.49	69.49	9.451	**
Error	399	2933.81	7.35		
Total	400	3003.31			

7.3.3. Recognition of cowpeas with different egg loads

The percentages of eggs laid on cowpeas with 1,2 or 3 eggs already attached is shown in Fig. 7.6. The percentages of eggs laid on each of the three types are not significantly different from each other.

7.4. Discussion

Avidov *et al* (1965b) found that *C. chinensis* females, when ovipositing on seven different sizes of steel balls, preferred intermediate sizes of steel balls to the largest or smallest sizes. Females preferred three sizes of steel balls between 3.18 and 6.35 mm in diameter (from a range of steel balls which were 2.38 to 15.88 mm in diameter). In the present study it was found that *C. maculatus* females apparently preferred an intermediate size of glass beads for oviposition; this was whether females were presented with a choice of medium-sized glass beads with one of the other sizes of glass beads or with both of the other sizes (Figs. 7.1 - 7.3). The results from this study and that of Avidov *et al* (1965b) were therefore similar.

The small glass beads have a smaller surface area than the medium-sized ones. Where only small and medium-sized glass beads were offered females may have laid eggs at random on any portion of bead surface (disregarding the size of bead) so that more eggs would be laid on the medium-sized beads than on the small ones.

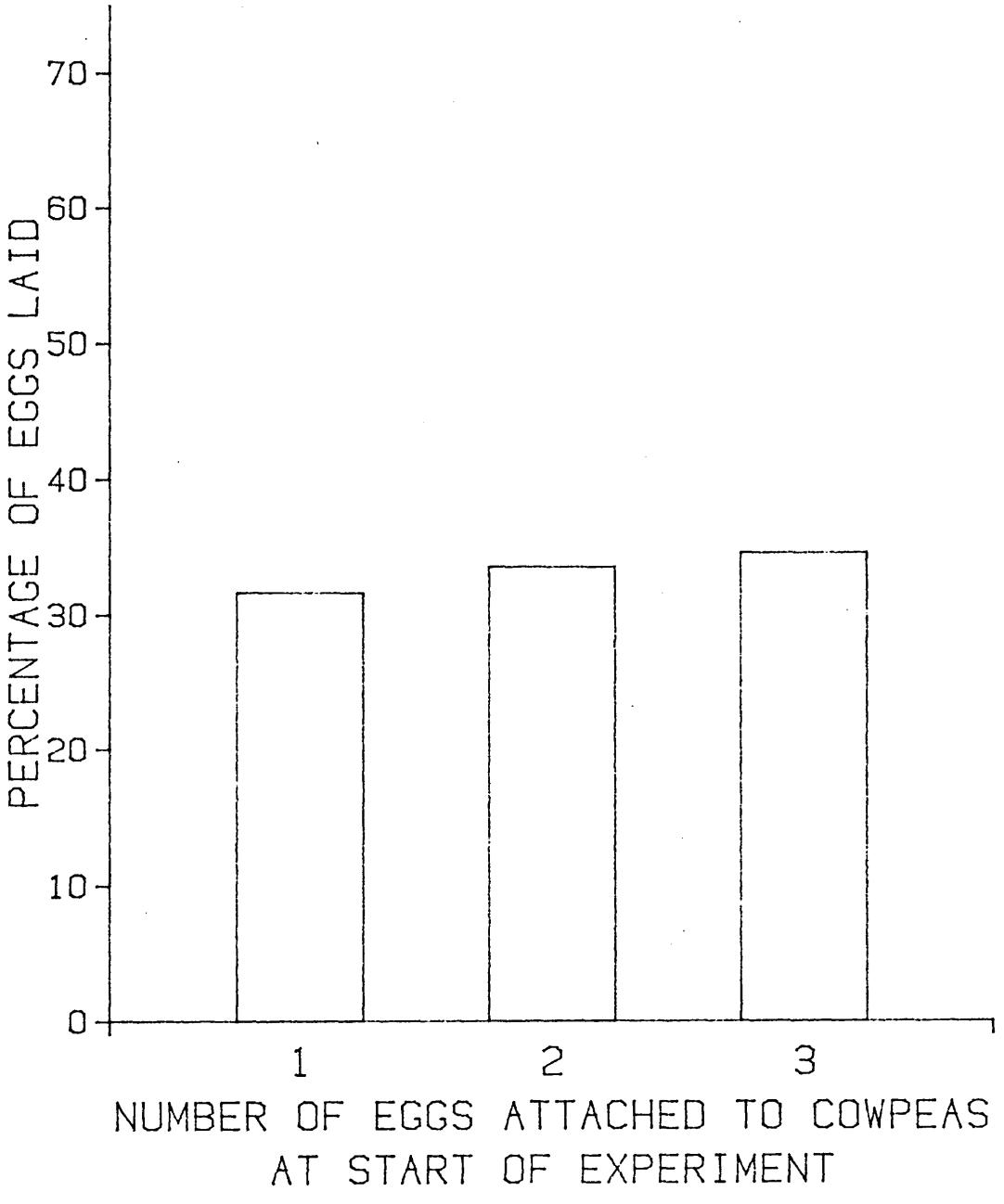


Figure 7.6: Effect of the presence of eggs on oviposition.

The percentage of eggs laid (average for twenty replicates) over 24 hours by newly emerged and mated Campinas females on a choice of fifteen cowpeas, five each with 1, 2 or 3 eggs already attached.

This theory matches the result. The ratio of the surface area of the small glass beads to the surface area of the medium-sized glass beads was approximately 1 to 3 and the ratio of the number of eggs was similar (approximately 1 to 2.5). Thus, the distribution of eggs laid on small and medium-sized glass beads could be explained by random oviposition. A similar explanation would not apply in the case of medium and large glass beads, however, since females clearly preferred the smaller of the two sizes. Similarly the female beetles might have specifically chosen to oviposit more eggs on the medium-sized beads than on the small ones but this cannot be distinguished.

The reason given by Avidov et al (1965b) for the observed behaviour of C. chinensis females was that the size of ball preferred closely corresponded to that of seeds for which the beetle displayed a marked preference. The medium-sized glass beads of the present study were the closest of the three sizes to cowpeas. It may be that both C. maculatus and C. chinensis females have an innate predilection for an oviposition substrate of a size which corresponds to that of their hosts, this would be of obvious value to a female searching for suitable oviposition sites.

Although C. maculatus females may reject an oviposition substrate which is above (or below) a certain size as being unsuitable for oviposition it is widely reported that they prefer the larger of a given species

or variety of seed (Mitchell, 1975; Nwanze & Horber, 1975; Nwanze et al, 1975). The results presented here indicate that this might only be the case at certain egg densities. At low densities of beetles (five and ten pairs), and consequently at low egg densities, there was no relationship between the weight of a cowpea and the number of eggs which were laid on it. This seems logical since it matters little to the survival of a female's offspring whether a cowpea will support ten or fifteen individuals if only one or two eggs are laid on every cowpea. Therefore one would not expect selection for females with a strategy which required them to choose larger cowpeas at low egg densities; in fact, one might expect that the females which chose larger cowpeas at this stage would be selected against since they would waste time and energy.

At higher densities of beetles (forty or two hundred) egg density was greater and there was a significant correlation between the weight of a cowpea and the number of eggs which were laid on it (Figs. 7.4 and 7.5). As the number of eggs on a cowpea increased so a point was reached at which the addition of any further eggs was likely to cause a marked rise in larval mortality (Credland et al, 1986). It would benefit a female to avoid ovipositing on cowpeas whose egg loads (and hence larval density) approached this maximum (the maximum that a cowpea could be expected to support) in favour of cowpeas whose egg loads were lower. If females

did not discriminate between small and large cowpeas at first, then the number of eggs on smaller cowpeas would approach the maximum before the number of eggs on larger cowpeas did. At this stage it would benefit females to begin avoiding smaller cowpeas in favour of larger ones since the bigger cowpeas can support more larvae than smaller ones (Dick, 1984). This would therefore explain the observed behaviour of C. maculatus females.

Analysis of covariance (Table 7.3) showed that the regression coefficients (Table 7.2) of the results for 20 pairs and for 200 unsexed beetles are significantly different. Females of the 200 unsexed beetles laid proportionately more eggs on larger cowpeas than did the 20 females. Egg density on the cowpeas which 200 beetles were on was much greater that on the cowpeas 20 pairs were on (9.03 eggs per cowpea compared to 2.56 eggs per cowpea) and so smaller cowpeas were much more likely to bear the maximum which they could be expected to support, making it even more beneficial for females to avoid ovipositing on them. As egg density increased, more cowpeas would support the maximum number of larvae that they could be expected to and so the benefit to be obtained by a female which discriminated between cowpeas on the basis of size would also increase.

What sort of strategy might be responsible for such an outcome? If a female could estimate the number of eggs on a cowpea and relate this to the maximum number

that the cowpea could be expected to support then it could oviposit or not depending upon whether the 'maximum' number of eggs were attached to a cowpea. This hypothesis probably ascribes to C. maculatus far more ability than it possesses. There is no evidence that females can actually 'count' the eggs on a cowpea or on any other bean; rather they can identify the bean which has the least number of eggs (Messina & Renwick, 1985a). To devise a strategy which would explain the behaviour of C. maculatus it might not even be necessary for a female to be aware of the absolute size of the cowpea upon which it rests but merely for it to be able to compare the size of one cowpea with another and to distinguish which is biggest.

Neither is it necessary for a female to evaluate (innately or otherwise) the egg load on a cowpea or to assess how many further eggs that cowpea might be expected to support, a more simple strategy might result in the efficient distribution of eggs. Mitchell (1975) hypothesised that in choosing whether to oviposit or not, a C. maculatus female would first estimate whether the bean it rested on was smaller or larger than the previous bean. If the bean was smaller then a female would only oviposit if the it had fewer eggs on it than the previous one; if the bean was larger or equal in size to the previous one then the female would oviposit unless the bean had more eggs than the previous one. Mitchell found that this model came close to explaining the observed

results for oviposition behaviour of C. maculatus on mung beans.

On mung beans only two or three eggs per bean are required to reach the maximum number of larvae which a bean can bear and so the weight of a mung bean is important even at low densities. Mitchell's model does not explain the oviposition behaviour of C. maculatus on cowpeas in its original form since at low egg densities the weight of a cowpea does not influence oviposition. If the sensitivity of the female to the difference in weight of the two cowpeas increased with increasing egg density then the resulting model might explain the behaviour of C. maculatus on cowpeas and other large beans. An increasing amount of oviposition deterring pheromone might raise the sensitivity of C. maculatus females to differences in cowpea size.

In order that this hypothesis remains feasible it is necessary to demonstrate that females can distinguish between cowpeas with different egg loads. The experiment in which females were presented with cowpeas with one, two and three eggs was designed to test this ability.

Messina & Renwick (1985a) stated that C. maculatus females could detect small differences in the number of eggs on separate black beans (Phaseolus vulgaris L.). The experiment was very similar to that of this study, apart from the fact that black beans were used. In the present study, however, females did not lay significantly

different numbers of eggs on the three classes of cowpea (Fig. 7.6), in other words the females apparently did not distinguish between cowpeas with different egg loads.

The theory that C. maculatus might be able to distinguish between small differences in egg load on black beans but not on cowpeas seems tenuous and a more likely explanation of the results lies in the design of the experiment. The cowpeas with 1,2 and 3 eggs were all taken from the same batch of cowpeas onto which 350 beetles had been placed. This means that these were cowpeas which females had already chosen to lay on once, twice or three times. It is entirely possible that cowpeas with three eggs attached had been favoured for, say, their smoothness, whilst those with only one egg were less attractive. The reason for which the cowpeas with most eggs were favoured may have counteracted the effect of the extra eggs, which would probably discourage oviposition, when these cowpeas were offered for choice with the cowpeas with fewer eggs. Thus, a female may have found the cowpeas with three eggs as suitable for oviposition as those with one, but for different reasons.

If this experiment were repeated, cowpeas with different egg loads should be collected from different sets of cowpeas with different egg densities. Thus cowpeas with three eggs would be collected from cowpeas whose average egg density was three eggs per cowpea, cowpeas with two eggs would be collected from cowpeas

whose average egg density was two eggs per cowpea and so on; in this way physical factors such as seed-coat smoothness would be negated. It would also be valuable to make the choice presented to the female more simple, say, between cowpeas with no eggs and cowpeas with one egg or between cowpeas with one egg and cowpeas with two eggs.

The studies of the oviposition strategy of C. maculatus presented in this chapter and the previous one have posed as many questions as they have answered. A discussion of the overall strategy of C. maculatus is presented in the following chapter.

Chapter 8

GENERAL DISCUSSION

8.1 The reproductive biology of C. maculatus females and other bruchids.

In a discussion of the reproductive biology of Callosobruchus maculatus and other bruchids the adult stage cannot be divorced from the larval stage. Factors influencing larvae may not only increase larval mortality but also affect their fecundity as adults. Seed type has been shown to affect the fecundity of C. maculatus (El Sawaf, 1956) and Acanthoscelides obtectus females (Herford, 1935). Increased larval competition not only increases larval mortality (Bellows, 1982b) but, by reducing the weight of emerging adult females, also reduces their fecundity (Credland et al, 1986). Conditions during the larval stage may also trigger the development of the "active" form of C. maculatus, which is less fecund than the "normal" form (Messina & Renwick, 1985b), these include temperature and larval crowding (Sano, 1967). Thus, whilst this discussion is concerned primarily with the adult beetle, it should be remembered that the potential fecundity of bruchids varies considerably on emergence and is determined to some extent by the previous generation.

The adult life of the female is spent in trying to realise its potential fecundity. All of its efforts are bent towards reproducing or improving its chances of doing so. The fecundity of adult females is often measured in terms of the number of eggs it lays (indeed that has been of primary interest when fecundity has been studied here) but fecundity should reflect a female's real reproductive fitness and so the number of larvae and adults which the eggs produce are actually more important. A female which lays many eggs, but in unsuitable places so that they do not hatch, has a lower fecundity, in the sense used here, than a female which lays a few eggs which develop successfully.

Credland et al (1986) took this a stage further, arguing that it is the number of eggs that a female's offspring produce (or fertilise, in the case of males) which are a measure of that female's fecundity. In trying to maximise its fitness a female should not necessarily attempt to produce the maximum number of offspring possible but should distribute its eggs in such a way that the potential fecundity of its offspring is maximised. This should always be borne in mind when attempting to explain the behaviour of C. maculatus females.

Emergence and mating.

Once emerged, in order to reproduce successfully, a female must mate and find suitable seeds on which to lay its eggs. In a store which is heavily infested the meeting of males and females is almost inevitable but in the field or in lightly infested stores such chance meeting may be insufficient to ensure adequate fertilisation of the female population. To encourage successful mating, C. maculatus females emit a pheromone which excites males and thus increases the chances of their meeting (Qi & Burkholder, 1982). Although mating is clearly necessary (virgins do not lay fertile eggs), repeated matings do not seem to affect the fecundity of females despite the fact that they will readily mate a second time (Chapter 5). A single mating is also sufficient to permit normal multiplication by A. obtectus females (Labeyrie, 1964) although males which have mated several times produce less offspring from later matings (Labeyrie, 1966).

Finding the host-plant.

Without beans to lay them on, the fertile eggs of a female contribute nothing to its fitness, so a major concern of the female is to find a suitable host. Larson & Fisher (1938) reported that C. maculatus and A. obtectus females find beans by their odour but there are few references to any direct experimental evidence of

this. Jarry (1981) reported that A. obtectus females could locate plots of cowpeas isolated in the centre of cornfields, over 500m away from possible sources of infestation.

The search for suitable host-plants and seeds often leads to the dispersal of bruchids from the site of their emergence (Larson & Fisher, 1938; Jarry, 1981) and here the "active" form of C. maculatus has a particularly important role. Adult crowding increases the numbers of "active" adults produced (Taylor, 1974), although the relationship between crowding and the number of "active" adults produced is not simple (Messina & Renwick, 1985b); crowding of the "normal" form, as seen in old cultures, also seems to encourage flight (pers. obs.). Taylor and Agbaje (1974) found that most of the flying adults in a Nigerian store were of the "active" form. Taylor and Aludo (1974) took sweep-net samples in the field and found that the "active" form was predominant; they concluded that "active" females were principally responsible for the establishment of infestations in the field. However, the normal form is also capable of flight (pers. obs. and Messina & Renwick, 1985b) and its ability to infest new supplies of seeds must not be ignored.

The dispersal of bruchids is obviously important since it plays a major role in the infestation of both field crops and stored pulses. Little work has been done

on flight in C. maculatus; temperature seems to be an important stimulus (Larson & Fisher, 1938; Taylor & Agbaje, 1974) but information, such as the distances which females can fly, is lacking.

How would a female recognise suitable seeds if it landed on them and what triggers oviposition? Chemical stimuli seem likely to provide the most important cues for both of these functions, the same stimuli possibly serving both purposes. Whilst C. maculatus females will lay eggs on clean glass beads, the level of egg laying is lower than normal. However, females can be induced to lay a normal number of eggs by coating the beads with host-plant extract (Chapter 4). This also applies to A. obtectus (Monge, 1983) suggesting that chemical stimuli enable female bruchids to recognise a substrate as suitable for oviposition. Whether these chemical stimuli can be detected at a distance (and thus guide the female to the host-plant) or whether they are only detected on contact is unclear.

In the bruchids A. obtectus and Z. subfasciatus the presence of the host-plant is important in triggering both the development of oocytes and the onset of oviposition (Huignard, 1979; Pimbert & Pierre, 1983). The same is known to be true in C. maculatus but the function of host-plant stimuli beyond these bare facts is less clear; for instance it seems that there is no quantitative relationship between the amount of

host-plant extract and the number of eggs produced and laid by females. The host-plant extract seems just to trigger the onset of oviposition (Chapter 4).

The size and shape of seeds might also help the females to recognise them as suitable. Avidov et al (1965b) stated that C. chinensis preferred steel balls of a particular size which corresponded to that of its usual host, rejecting others which were markedly different in size. C. maculatus similarly rejected glass beads which were larger than its usual host (Chapter 7).

Despite the fact that some bruchids were able to find seeds at great distances (Jarry, 1981) there appears to be no relationship between the preference of females for a particular species of seed and its suitability for development. Under experimental conditions C. maculatus showed a preference for soyabean seeds as an oviposition site when offered a choice of several species of seeds despite the fact that fewer beetles were able to develop in soyabeans than in the other species which were offered (Girish, Singh & Krishnamurthy, 1974). This preference for unsuitable seeds is also found in C. chinensis (Morimoto, 1939; Ishii, 1952; Srivistava & Bhatia, 1959; Teotia & Singh, 1966). One can imagine, however, that there would be considerable selective pressures acting against such behaviour outside the laboratory so that females would soon begin to discriminate between those seeds which were suitable for the development of larvae

and those which were not (or the larvae might adapt to develop in such seeds).

The choice of oviposition site.

Assuming that the female has been successful in both mating and finding an oviposition site, the question then arises as to whether it will oviposit or not and if so, on which of the seeds? The combination of mating and the presence of host-plant seeds normally triggers oviposition in several species of bruchids (Huignard, 1979; Pimbert & Pierre, 1983) but certain conditions might alter this.

The presence of many eggs on individual seeds decreases the egg laying of C. maculatus females (Chapter 3) but outside the laboratory it might be that females would be discouraged from laying at all and leave to search for other, more suitable seeds. It is only in exceptional circumstances that females would actually be trapped in a small space with few seeds, although in practice a female may frequently have only a few cowpeas in its immediate vicinity. Whether females on a restricted number of cowpeas, which possibly already bore eggs, would opt to depart in search of a more suitable supply of cowpeas (with the chance that it may find no cowpeas at all) or whether it would oviposit on the present cowpeas (and suffer a reduction in its realised fecundity) probably depends on a balance of factors.

What potential do the present cowpeas have for reproduction, i.e. how many more larvae could be sustained and with what resulting fecundity? What energy reserves does the female have for flight? Etc.

Trapping females on a limited number of beans, as in laboratory experiments (although a conceivable natural occurrence - Chapter 3), must necessarily encourage behaviour which represents an attempt to maximise potential fecundity in the poorest of circumstances and which would not normally be seen elsewhere. Only carefully conducted field trials, without restrictions on dispersal, could properly test the reaction of females to heavily infested seeds since this would allow them to behave in a more natural way.

Also important in this respect is whether the seeds are unshelled or not, because the pod may deter egg laying by C. maculatus. Caswell (1968) stated that local farmers in Nigeria often leave cowpeas in their pods because this offers some degree of protection from attack by C. maculatus. Although C. maculatus larvae emerging from eggs laid on the outside of a cowpea pod are able to penetrate through the pod and into a cowpea, females prefer to oviposit on exposed cowpeas rather than on undehisced pods (Alzouma, 1981).

A. obtectus is better adapted to attack seeds in undehisced pods than C. maculatus because the female can perforate the pod and lay its eggs inside (Biemont & Bonet, 1981). Once hatched, the larvae (which are more mobile than those of C. maculatus because the female does not stick the eggs to a particular seed) are able to move along the inside of the pod and select a seed which they then enter (Biemont & Bonet, 1981).

The fecundity of Z. subfasciatus females is low on undehisced pods (Pierre & Pimbert, 1981). This is because, like C. maculatus, the larva must bore through the pod and directly into a seed in order to develop - if the larva misses a seed then it will fall into the spaces between the seeds in the pod and die (Prevett, pers. comm.).

If the beetle decides to lay eggs on the mass of seeds, the next problem is how will it select individual cowpeas on which to lay its eggs? A great deal of work has gone towards explaining the oviposition behaviour of different species of bruchids (Ueno, 1954; Avidov, Applebaum & Berlinger, 1965; Avidov, Berlinger & Applebaum, 1965; Umeya, 1966; Nakamura, 1968; Umeya & Kato, 1970; Gokhale & Srivistava, 1975; Pouzat, 1983; Smith & Lessells, 1985) but perhaps the best hypothesis of oviposition strategy presented so far applies to C. maculatus (Mitchell, 1975). Mitchell's model is relatively straightforward and seems to adequately

explain the oviposition behaviour of C. maculatus on mung beans. The model was presented as a computer program which simulated the oviposition strategy of C. maculatus and is given in Table 8.1.

The present study has produced evidence to support Mitchell's model. C. maculatus females were able to distinguish between marked and control cowpeas more easily when the two types of cowpeas were mixed than when they were in groups in the choice chambers (Chapter 6) - this suggests that the females may compare adjacent beans. Females were also able to distinguish between glass beads or cowpeas of different sizes (Chapter 7), an important part of Mitchell's model.

Although there is evidence to support Mitchell's model in so far as it goes (which is to describe the oviposition behaviour of C. maculatus on mung beans), the model does not adequately describe the oviposition behaviour of C. maculatus on cowpeas. This is because at low egg (or adult) densities the females ignored the size of the cowpeas they were laying on (Chapter 7). Therefore any model which attempted to describe the strategy of C. maculatus on cowpeas must allow for an increase in discrimination between cowpeas of different sizes as the mean egg density increased.

Table 8.1: Mitchell's basic commands for a program simulating the oviposition strategy of C. maculatus

Previous bean smaller	Present bean larger	Present bean with fewer eggs → OVIPOSIT
		Number of eggs equal → OVIPOSIT
		Present bean with more eggs → Reject
Previous bean larger or equal	Present bean smaller	Present bean with fewer eggs → OVIPOSIT
		Number of eggs equal → Reject
		Present bean with more eggs → Reject

The complexity of the situation facing an ovipositing female is further increased when one considers that cowpeas are likely to have different egg loads and not simply to have eggs or not. Females, therefore, must be able to distinguish between cowpeas with different egg loads and there is evidence to support this (Messina & Renwick, 1985a). In the present study, increasing the strength of the egg marker appeared to enable females to more readily distinguish between marked and unmarked cowpeas (Chapter 6).

Another important influence on oviposition is the texture of the seed coat (Nwanze & Horber, 1976). This was ignored by Mitchell (1975), possibly because of the uniform smoothness of the seedcoat of mung beans. Any model of C. maculatus' oviposition strategy should explain the influence of seed coat texture on oviposition. Although it has been reported that C. maculatus preferred smoother seed coats for oviposition (Nwanze & Horber, 1976) this was not quantified, presumably because of the difficulty in measuring seed coat texture. The potential of the seedcoat to prevent infestation by C. maculatus has also largely been ignored even though harder and thicker seedcoats have been shown to cause the death of larvae before they can enter the seed (Janzen, 1977).

For C. maculatus to distribute its eggs most favourably it is important that females can recognise seeds with eggs already on them and act accordingly. In all of the bruchid species in which this has been investigated the method of recognition appeared to be at least partly by means of pheromones (Yoshida, 1961; Oshima, Honda & Yamamoto, 1973; Giga & Smith, 1985; Szentesi, 1981). These pheromones were deposited by both male and female beetles when walking over beans, though egg laying may have enhanced its production by females. They had the effect of deterring subsequent oviposition.

The pheromones have been described as 'egg markers' (Oshima et al, 1973) but this cannot apply to those which were deposited by males or by virgin females - a better term, which can be applied to them all because it describes a common function, though not necessarily the only function, is 'oviposition deterring pheromones'.

The oviposition deterring pheromone of C. maculatus has been shown to cause an overall reduction in oviposition by females in some circumstances (Giga, 1982). In the present study, however, females presented with no choice but to lay on cowpeas coated with pheromone did not reduce their egg laying when compared with females on uncoated cowpeas (Chapter 6). It is possible that the oviposition deterring pheromone served to delay the onset of oviposition for a short time when females were on marked cowpeas only - after this delay,

which possibly served to encourage females to search for unmarked cowpeas, the female laid on the only cowpeas available in the vicinity, i.e. marked cowpeas.

Over a longer period one function of the oviposition deterring pheromone may be to allow females to distribute their eggs more advantageously. Where there are a number of cowpeas with different egg loads it is best that a female avoids those cowpeas which bear the greater number of eggs.

This is very much a simplified appraisal of the way in which oviposition deterring pheromones function though. To begin with the eggs may be those of different females and this may affect the way in which a female responds to them because it might benefit a female to reduce the potential fecundity of offspring other than her own; Smith & Lessells (1985) discussed the advantages of ovicidal behaviour in bruchids but actual evidence for such behaviour is tenuous (Chapters 5 & 6). It is probable, however, that C. maculatus females cannot tell their own eggs from those of other females since this is apparently the case with C. chinensis (Avidov et al, 1965a).

Once a female has laid its eggs on a number of cowpeas and left in search of other cowpeas, or died, the advantage to be gained by ensuring careful distribution of eggs on these cowpeas remains because other females may arrive to oviposit on them. It is of benefit to the

offspring of both the original and subsequent females that eggs continue to be efficiently distributed on the cowpeas by other females because larvae at different stages of development can interfere with each other (Bellows, 1982b). Therefore it is important that the pheromone deposited by the original female remains effective long enough to provide information about the presence of eggs whilst developing beetles remain within the cowpeas. The pheromone of C. maculatus does have a lasting effect (Chapter 6), long enough to allow the complete development of C. maculatus from egg to adult under the experimental conditions of this study.

C. maculatus females are able to recognise seeds bearing the eggs of at least two other species of bruchids (C. chinensis and C. rhodesianus) and vice versa (Yoshida, 1961; Giga, 1982) bringing yet another dimension to the choices facing an ovipositing female. The consideration a female gives to eggs of its own species and those of another when ovipositing must firstly depend upon its ability to distinguish between eggs of different species and on the advantages and disadvantages of laying eggs on seeds already infested by bruchids of a different species. Prokopy, Reissig & Moericke (1976) found that flies of the genus Rhagoletis were able to recognise the eggs of other species of the group and that the more closely related a female was to a species then the more likely it was that it would recognise and avoid the eggs of that species.

Overall, the oviposition deterring pheromone of C. maculatus, and those of other bruchids, seem well adapted to their function of ensuring the efficient distribution of each species' eggs over the seeds available.

The phrase 'efficient distribution of eggs' has been used here in preference to 'uniform distribution of eggs' for two reasons. Firstly because in this study C. maculatus did not always distribute its eggs uniformly even on fresh cowpeas, and secondly because the uniform distribution of eggs (an even number of eggs on each cowpea) is not necessarily the most efficient (maximising the potential fecundity of the female's offspring).

C. maculatus females have been shown in this study to avoid cowpeas which are marked with oviposition deterring pheromone (Chapter 6). They have also been shown to prefer to oviposit on cowpeas with the least number of eggs on them (Messina & Renwick, 1985a). One might expect, therefore, that beetles with such abilities would tend to distribute their eggs in a uniform manner since they would always choose to oviposit on cowpeas with the least number of eggs on them. However, throughout this study C. maculatus females did not distribute their eggs uniformly but appeared to distribute them randomly. There are two possible reasons for this.

The females may indeed have oviposited on cowpeas at random, taking no regard of the number of eggs already on a cowpea or of any other characteristic of the cowpeas beyond ensuring that they actually were ovipositing on cowpeas and not, say, the glass tubes.

Initially at least, there might be no need for females to distribute their eggs uniformly. A single cowpea can support several larvae of most geographical strains (Dick, 1984) and so, on fresh cowpeas, a female might at first oviposit at random, with little danger of laying sufficient eggs on any one cowpea to cause larval overcrowding. The advantage which a female might gain from random oviposition on fresh cowpeas is that it would not waste time unnecessarily searching for cowpeas with no eggs. This would mean that the offspring of a female could begin development before those of other females (which might be expected to arrive at any time) and so avoid some competition.

Only when the egg load on the cowpeas began to build up (and this could be detected by the build up of oviposition deterring pheromone) might the female gain an advantage by ovipositing more selectively. There is no evidence, however, of any relationship between the tendency towards a uniform distribution of eggs and the density of eggs on the cowpeas (pers. obs.).

The second possible explanation for the apparently random distribution of eggs by C. maculatus females lies in the cowpeas themselves. Every reasonable effort was made to ensure that the cowpeas used in experiments were as similar as possible but inevitably there were still slight variations between them. These variations included the texture of the seed coat (which varied from almost glass-like smoothness to that of fine sandpaper), the size of cowpeas (despite removing the smaller ones by sieving) and their shape. It is also quite possible that the cowpeas differed from each other in ways which are not readily perceptible to the human senses, for instance the chemical constituents of the seed coat may vary from cowpea to cowpea.

Such slight variations between cowpeas might significantly affect the choice of oviposition site by C. maculatus females so that, whilst appearing to oviposit randomly, they were in fact ovipositing selectively.

When the variations in the oviposition substrate was minimal, selective oviposition would tend to result in a uniform distribution of eggs. This may be the reason for the uniform distribution of eggs of C. maculatus on mung beans (Mitchell, 1975) which are uniformly smooth in texture. If the variety of cowpea used by Gokhale & Srivistava (1975) was a smooth textured one then the uniform distribution of eggs laid by C. maculatus which

they observed could also be explained by selective oviposition.

Pouzat (1983) reported that A. obtectus oviposited randomly on Phaseolus vulgaris seeds and whilst most workers describe C. chinensis as distributing its eggs uniformly (Ishii, 1952; Avidov et al, 1965a; Nakamura, 1968), others have stated that the females of this species oviposit randomly in some circumstances (Utida, 1943; Ueno, 1954). Perhaps this apparent anomaly can be explained by the concept of selective oviposition, the apparently random distribution of eggs being due to the beetles selecting particular seeds and rejecting others for reasons which were not clear at the time and which were not catered for in the experimental design.

Clearly more work needs to go into the effect of seedcoat texture on oviposition strategy. This has probably been greatly hampered by the difficulty involved in quantifying the smoothness of seedcoats.

It should be stressed that the variations among the cowpeas, though disturbing the uniform distribution of eggs, would not affect the validity of the results presented in this study because of the design of the experiments. Slight variation between cowpeas would not affect the results from, for example, choice chamber experiments because both marked and control cowpeas would, on average, present the same degree of variation.

Feeding and energy reserves.

Females 'in the field' can feed on pollen and if they do so this increases both their life-span and fecundity (Larson & Fisher, 1938). Females in store do not feed however (Dobie, 1981) and so all their energy reserves are present at emergence - mainly in the fat body. Under these circumstances the decline in egg laying (which is at a maximum on the day of emergence in ideal conditions) corresponds closely to the decline in the energy reserves of the female (Utida & Takahashi, 1958; Caswell, 1960; Sidhu et al, 1980; Sharma et al, 1983; Puri & Sharma, 1984 and Chapter 3). Other species of bruchids may also feed as adults thereby increasing their life-span and fecundity. These include C. chinensis (Williams, 1977) and A. obtectus (Leroi, 1981). Females of Bruchus pisorum L. are unusual in only being able to reproduce after consuming pollen of their host-plant, Pisum sativum L. (Panji & Sood, 1975).

8.2 Strain differences.

So far in this discussion, strain differences have largely been ignored. Most work presented here concerns the Campinas strain of C. maculatus. It was not possible to include the other two strains in every experiment because of the limited time available.

Dick (1984) showed that the IITA strain did not suppress its egg laying when restricted to a few cowpeas whereas females of the other two strains did. The present study confirmed these results, indicating that, when provided with two cowpeas only, females of the Campinas and Yemen strains first retained eggs in their oviducts and then decreased egg production, but females of the IITA strain continued to produce and lay as many eggs as did those on 40 cowpeas (Chapter 3). A possible reason for this is that the potential fecundity of an IITA female's offspring (calculated by extrapolation from a series of different experiments) did not diminish with increased larval crowding (over the range of larval density studied); for females of the Campinas and Yemen strains, as larval density increased, the estimated potential fecundity of their offspring reached a maximum and thereafter may have decreased (Credland et al, 1986). This means that the IITA females were able to continue laying eggs on two cowpeas, whereas the Campinas and Yemen females suppressed their egg laying, without decreasing the total potential fecundity of their offspring.

A further example of strain differences can be found in the differing abilities of larvae to develop in cowpeas. Cowpeas which might be expected to produce ten or twelve adults of the Campinas or IITA strains only supported the development of two or three Yemen individuals (Dick, 1984). The reason for this was not

clear but it might be expected to cause important differences in the reproductive behaviour of the Yemen strain and that of the other two strains. For instance, there was subjective evidence of a difference in the way that females of the different strains reacted to cowpeas coated with the oviposition deterring pheromone. Yemen females appeared to avoid ovipositing on marked cowpeas to a greater extent than females of the other two strains (Chapter 6). In addition to these differences is the manner in which the strains differed in their ability to develop on the resistant variety of cowpea, TVu 2027 (Dick, 1984).

In view of the instances already found it seems reasonable to suppose that strain differences might affect any aspect of the reproductive biology of C. maculatus to varying degrees. Thus, although a particular problem may be solved using one strain, the solution does not necessarily apply to other strains and when one considers that C. maculatus is a cosmopolitan species with a considerable potential for geographical variation, it becomes evident that any attempt to develop new methods of control must also deal with the problems caused by strain differences.

8.3 Conclusions

When studying a beetle such as C. maculatus in the laboratory, or even in store, it is sometimes easy to forget that it initially evolved in the African tropics (Southgate, 1979). One should always remember that the circumstances of an animal's evolution must be reflected in its biology. Behaviour that seems anomalous in the laboratory, such as the way in which IITA females did not suppress their egg laying (Chapter 3), almost certainly evolved for good biological reasons in the first instance.

A study of the reproductive biology of C. maculatus is of value for both academic and practical reasons. Population modelling is a useful tool which aims to predict long term changes from data collected over a relatively brief period. C. maculatus is an excellent species for such studies because it is very easy to maintain in culture and has a short life-cycle in which the adult stage does not feed and the immature stages pass within a single seed. In developing models which predict the behaviour of an animal population under given circumstances it is important that one has a sound knowledge of the biology of that species (its abilities and limitations) since this increases accuracy.

Mitchell (1975) was able to incorporate the effect of bean size into his model of the oviposition strategy of C. maculatus on mung beans. The present study showed that this model does not sufficiently describe the oviposition behaviour of C. maculatus on cowpeas because egg (or adult) density appears to influence the ability (or readiness) of females to discriminate between cowpeas of different weight (Chapter 7). With this increased knowledge of the biology of C. maculatus one can attempt to adapt Mitchell's model to cowpeas by allowing for the influence of egg density.

This study has also given an indication of the way in which models predicting, say, the oviposition strategies of C. maculatus females might be developed to cope with more realistic circumstances. Whether a female continues to oviposit on cowpeas which already have several eggs on them will probably depend on how such behaviour is likely to affect the potential fecundity of that female's offspring (Chapter 3) and this in turn might affect the design of a model of oviposition strategy. Thus, increased knowledge of the biology of C. maculatus allows workers to increase the accuracy and sophistication of their models and thereby their usefulness.

For the same reason that C. maculatus is so valuable for studying population biology and ecology it is also a serious pest. Its ability to multiply rapidly causes

serious damage (in Nigeria alone, this is estimated at £20 million per year (IITA, 1983)). This provides the second and most important reason for studying the reproductive biology of C. maculatus. With increased knowledge of the subject it becomes possible to suggest methods of control which might be suited to developing countries where damage is most serious and where it can least be afforded. If one can break the cycle of reproduction then the damage caused by this pest would be reduced. This might be achieved by removing a necessary stimulus for oviposition, by disturbing efficient oviposition or by decreasing the chance of an egg developing into an adult. There are several suggestions as to how this might be achieved arising from this study.

It was suggested that the oviposition deterring pheromone of C. maculatus might be used to protect cowpeas (Chapter 1) but it soon became clear that such a simple method of control would not be successful because females will oviposit on marked cowpeas when no others are available (Chapter 6). This does not mean that the oviposition deterring pheromone has no potential as a control method, since it might, under normal circumstances, encourage females to disperse from cowpeas before they oviposit. This is worthy of further investigation.

C. maculatus prefer soyabeans to cowpeas for oviposition even though the former are less suitable for the development of larvae (Girish et al, 1974). Mixing cowpeas and soyabeans together when in store might protect the cowpeas from attack by C. maculatus since females would tend to lay their eggs on the soyabeans. Marking the cowpeas with oviposition deterring pheromone might decrease egg laying on the cowpeas still further.

The seedcoat of cowpeas offers considerable potential for the control of C. maculatus. The seedcoats contain chemical components which stimulate oviposition (Chapter 4) and it might be possible to breed cowpea varieties without these components making them less likely to be recognised by C. maculatus females as suitable for oviposition.

Rough coated varieties of cowpeas are less attractive for oviposition by C. maculatus than smooth coated ones (Nwanze & Horber, 1976) and thicker, harder seedcoats prevent larvae from entering seeds (Janzen, 1977). If cowpeas could be bred with hard, rough coats then this might again prove an effective barrier to attack. Such a method would have a distinct advantage in that the cowpeas with hard, rough coats would lose none of their nutritional value, as might cowpeas which were bred with altered chemical components.

These methods of control have the advantage that, once established, they could be applied by farmers in developing countries at little or no expense on their part and without the need for complicated practices to be adopted.

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Environ. Entomol., 8: 277-279.

APPENDIX 1

Composition of Beetle Ringer

Taken from:

Ramsay, J.A. (1964)

The rectal complex of the mealworm
Tenebrio molitor (Coleoptera, Tenebrionidae).

Phil. Trans. R. Soc. Ser. B, 248: 279-314.

Composition:

NaCl	3.8g
Na_2HPO_4	1.4g
KCl	4.1g
$CaCl_2$	1.1g
$MgCl_2.6H_2O$	2.0g
Water	1000ml

The precipitate of earthy phosphate was filtered off and the filtrate brought to pH 7.1.

APPENDIX 2

Weight Loss on Total Egg Laying ,Change in Oocyte Number with Age and Daily Egg Laying.

a) Campinas

Age When Killed	Orig Weight (mg)	Final Weight (mg)	Total Number of Eggs Laid Each Day 1	2	3	4	5	6	7	Tot. Eggs Laid	Total No. of Oocytes
2 Hrs	4.225	4.000	6	-	-	-	-	-	-	6	35
"	3.015	2.902	3	-	-	-	-	-	-	3	37
"	3.207	3.082	2	-	-	-	-	-	-	2	37
"	4.044	3.933	2	-	-	-	-	-	-	2	35
"	3.361	3.324	0	-	-	-	-	-	-	0	40
"	4.421	4.226	8	-	-	-	-	-	-	8	--
"	2.900	2.889	0	-	-	-	-	-	-	0	--
"	4.146	3.971	0	-	-	-	-	-	-	0	--
"	3.523	3.496	0	-	-	-	-	-	-	0	--
"	4.865	4.680	0	-	-	-	-	-	-	0	--
4 Hrs	2.748	2.516	4	-	-	-	-	-	-	4	22
"	4.245	3.999	5	-	-	-	-	-	-	5	38
"	4.314	4.159	0	-	-	-	-	-	-	0	--
"	5.854	5.771	0	-	-	-	-	-	-	0	--
"	5.369	5.109	4	-	-	-	-	-	-	4	36
"	4.732	4.558	3	-	-	-	-	-	-	3	29
"	4.666	4.395	4	-	-	-	-	-	-	4	33
"	4.446	4.358	0	-	-	-	-	-	-	0	--
"	4.918	4.923	1	-	-	-	-	-	-	1	--
"	5.196	5.053	1	-	-	-	-	-	-	1	--
1 Day	4.207	3.705	16	-	-	-	-	-	-	16	32
"	6.318	5.446	15	-	-	-	-	-	-	15	46
"	4.865	4.158	22	-	-	-	-	-	-	22	38
"	4.953	4.256	17	-	-	-	-	-	-	17	40
"	5.000	4.348	18	-	-	-	-	-	-	18	36
"	5.655	4.900	24	-	-	-	-	-	-	24	--
"	3.995	3.404	12	-	-	-	-	-	-	12	--
"	5.306	4.552	17	-	-	-	-	-	-	17	--
"	4.014	3.755	18	-	-	-	-	-	-	18	--
"	5.099	4.855	20	-	-	-	-	-	-	20	--

a) Campinas contd.

Age When Killed	Orig Weight (mg)	Final Weight (mg)	Total Number of Eggs Laid Each Day 1	2	3	4	5	6	7	Tot. Eggs Laid	Total No. of Oocytes
4 days	5.829	3.959	20	23	15	10	-	-	-	68	27
"	4.461	3.570	-	-	17	5	-	-	-	22	25
"	4.401	2.631	28	22	11	10	-	-	-	71	18
"	4.306	2.654	17	18	15	7	-	-	-	57	22
"	4.149	2.825	13	16	9	10	-	-	-	48	22
"	5.216	3.525	25	18	12	7	-	-	-	62	--
"	4.572	3.164	15	17	15	10	-	-	-	57	--
"	5.265	3.394	22	22	16	10	-	-	-	70	--
"	4.866	4.292	-	-	-	1	-	-	-	1	--
"	5.214	3.510	21	23	17	11	-	-	-	72	--
6 days	4.512	2.839	8	16	16	13	13	8	-	74	16
7 days	4.377	2.452	?	14	15	11	10	5	0	?	12
"	3.593	?	6	15	14	15	10	6	X	66	10
"	3.715	?	1	11	16	14	2	0	X	44	12
"	3.898	2.162	?	15	16	12	11	4	5	?	10
"	3.182	1.737	5	9	12	9	5	0	0	40	--
"	3.916	2.805	0	0	0	0	0	0	0	0	--
"	4.629	2.551	1	14	16	18	15	12	5	81	12
"	5.023	3.645	0	1	0	1	0	12	8	22	--
"	4.590	3.015	0	0	0	0	0	0	0	0	--
"	3.976	1.945	14	16	20	13	11	8	4	86	--

X - Female Dead ? - Result Unknown

b) IITA

Age When Killed	Orig Weight (mg)	Final Weight (mg)	Total Number of Eggs Laid Each Day 1	2	3	4	5	6	7	Tot. Eggs Laid	Total No. of Oocytes
1 Hr	4.399	4.319	0	-	-	-	-	-	-	0	--
"	4.155	4.039	0	-	-	-	-	-	-	0	--
"	5.536	5.278	0	-	-	-	-	-	-	0	--
"	5.474	5.304	0	-	-	-	-	-	-	0	--
"	5.304	5.111	0	-	-	-	-	-	-	0	--
"	4.789	4.712	0	-	-	-	-	-	-	0	36
"	4.989	4.820	0	-	-	-	-	-	-	0	37
"	5.080	4.917	0	-	-	-	-	-	-	0	37
"	5.844	5.667	0	-	-	-	-	-	-	0	37
"	4.291	4.245	0	-	-	-	-	-	-	0	26
2 Hrs	2.757	2.745	0	-	-	-	-	-	-	0	24
"	6.142	6.097	0	-	-	-	-	-	-	0	44
"	5.279	5.161	0	-	-	-	-	-	-	0	37
"	3.119	3.098	0	-	-	-	-	-	-	0	21
"	4.944	4.673	0	-	-	-	-	-	-	0	29
"	5.936	5.863	0	-	-	-	-	-	-	0	--
"	5.247	5.071	0	-	-	-	-	-	-	0	--
"	5.023	4.972	0	-	-	-	-	-	-	0	--
"	?	?	0	-	-	-	-	-	-	0	--
"	5.462	5.412	0	-	-	-	-	-	-	0	--
4 Hrs	5.538	5.335	0	-	-	-	-	-	-	0	--
"	4.324	4.127	0	-	-	-	-	-	-	0	35
"	3.639	3.484	1	-	-	-	-	-	-	0	30
"	5.366	5.208	0	-	-	-	-	-	-	0	37
"	4.015	3.878	0	-	-	-	-	-	-	0	34
"	5.800	5.477	0	-	-	-	-	-	-	0	37
"	4.346	4.040	4	-	-	-	-	-	-	4	--
"	4.495	4.004	8	-	-	-	-	-	-	8	--
"	5.779	5.680	0	-	-	-	-	-	-	0	--
2 Days	5.148	4.081	21	21	-	-	-	-	-	42	36
"	4.795	3.871	20	16	-	-	-	-	-	36	36
"	6.194	5.001	25	21	-	-	-	-	-	46	44
"	5.428	4.192	14	18	-	-	-	-	-	32	29
"	4.706	3.669	17	17	-	-	-	-	-	34	47
"	5.540	4.207	17	16	-	-	-	-	-	33	--
"	4.724	3.896	18	17	-	-	-	-	-	35	--
"	5.119	4.225	2	24	-	-	-	-	-	26	--
"	4.511	3.566	23	16	-	-	-	-	-	39	--
"	6.095	4.768	17	18	-	-	-	-	-	35	--

b) IITA contd.

Age When Killed	Orig Weight (mg)	Final Weight (mg)	Total Number of Eggs Laid Each Day 1	2	3	4	5	6	7	Tot. Eggs Laid	Total No. of Oocytes
3 Days	5.363	3.709	18	20	19	-	-	-	-	57	40
"	5.548	3.808	22	23	20	-	-	-	-	65	51
"	5.046	3.576	20	23	21	-	-	-	-	64	36
"	3.971	3.008	10	17	10	-	-	-	-	37	--
"	5.216	3.751	18	25	17	-	-	-	-	60	36
"	5.759	4.247	14	20	20	-	-	-	-	54	35
"	6.201	4.584	14	21	21	-	-	-	-	56	--
"	6.210	4.548	9	25	19	-	-	-	-	53	--
"	5.779	4.255	18	17	13	-	-	-	-	48	--
4 Days	4.517	3.219	16	9	12	11	-	-	-	48	21
"	6.307	3.877	17	20	22	15	-	-	-	74	36
"	6.476	4.165	22	22	19	18	-	-	-	81	35
"	5.948	4.034	27	15	23	13	-	-	-	78	34
"	4.508	3.127	11	13	23	15	-	-	-	62	30
"	5.427	3.509	21	27	17	18	-	-	-	83	--
"	6.014	4.071	21	17	18	18	-	-	-	74	--
"	5.679	3.703	23	21	20	20	-	-	-	84	--
5 Days	4.558	2.437	21	17	16	12	8	-	-	74	14
"	4.680	2.496	19	15	19	20	13	-	-	86	23
"	4.169	2.467	22	15	12	21	6	-	-	76	16
"	3.874	2.487	15	15	12	12	2	-	-	56	18
"	2.877	1.804	10	13	12	11	6	-	-	52	11
"	2.748	1.631	16	0	24	9	6	-	-	55	--
"	3.576	2.222	19	14	9	8	10	-	-	60	--
"	3.705	2.093	10	15	16	10	6	-	-	57	--
"	4.273	2.439	22	14	17	14	6	-	-	73	--
6 Days	3.153	2.090	0	2	8	4	6	6	-	26	12
"	4.816	2.719	10	19	19	13	7	4	-	72	20
"	4.735	2.305	10	14	11	8	7	1	-	51	12
"	4.908	?	0	14	14	15	7	X	-	50	--
"	3.586	2.014	11	2	14	5	6	6	-	44	10
"	3.380	2.769	0	0	0	0	0	0	-	0	20
"	3.382	2.204	25	14	10	12	8	4	-	73	--
"	4.063	2.145	27	19	11	15	8	5	-	85	--
"	4.003	2.216	14	13	13	16	11	7	-	74	--

X - Female Dead, ? - Result Not Known

b) IITA contd.

Age When Killed	Orig Weight (mg)	Final Weight (mg)	Total Number of Eggs Laid Each Day 1	2	3	4	5	6	7	Tot. Eggs Laid	Total No. of Oocytes
7 Days	3.619	1.821	21	13	16	15	11	7	6	89	--
"	5.019	3.398	4	4	10	4	5	5	1	33	--
"	3.199	1.839	11	11	11	9	6	4	2	54	4
"	4.329	2.637	6	6	10	2	0	0	0	24	12
"	4.344	2.242	19	16	15	9	10	9	3	81	10
"	4.086	?	5	17	6	1	0	X	X	29	--
"	4.219	2.567	14	16	11	16	6	16	5	84	7
"	5.664	3.225	2	2	8	21	9	3	3	48	--
"	3.680	2.202	17	14	9	14	4	9	2	69	7
"	5.166	4.439	0	0	0	0	0	0	1	1	--

X - Female Dead ? - Result Not Known

c) Yemen

Age When Killed	Orig Weight (mg)	Final Weight (mg)	Total Number of Eggs Laid Each Day 1	2	3	4	5	6	7	Tot. Eggs Laid	Total No. of Oocytes
2 Hrs	9.796	9.621	0	-	-	-	-	-	-	0	30
"	7.160	6.992	0	-	-	-	-	-	-	0	34
"	9.494	9.398	0	-	-	-	-	-	-	0	--
"	7.450	7.207	5	-	-	-	-	-	-	5	26
"	8.545	8.427	0	-	-	-	-	-	-	0	40
"	11.247	11.178	0	-	-	-	-	-	-	0	34
"	7.385	7.388	0	-	-	-	-	-	-	0	--
"	10.488	10.463	0	-	-	-	-	-	-	0	--
"	10.804	10.703	1	-	-	-	-	-	-	1	--
"	11.972	11.888	0	-	-	-	-	-	-	0	--
1 Day	10.380	9.003	21	-	-	-	-	-	-	21	49
"	9.284	9.032	0	-	-	-	-	-	-	0	--
"	9.386	8.344	20	-	-	-	-	-	-	20	41
"	11.810	11.453	0	-	-	-	-	-	-	0	--
"	10.292	9.937	0	-	-	-	-	-	-	0	--
"	12.385	11.140	14	-	-	-	-	-	-	14	47
"	4.657	4.533	0	-	-	-	-	-	-	0	--
"	10.723	9.430	29	-	-	-	-	-	-	29	48
"	9.739	8.682	25	-	-	-	-	-	-	25	--
"	10.786	9.739	20	-	-	-	-	-	-	20	--
"	9.796	9.621	18	-	-	-	-	-	-	18	35
"	7.160	6.992	12	-	-	-	-	-	-	12	37
4 Days	6.223	4.001	20	17	-25-		-	-	-	62	25
"	9.855	6.669	5	30	-44-		-	-	-	79	25
"	11.556	7.847	31	19	-42-		-	-	-	92	37
"	9.864	6.465	24	23	-25-		-	-	-	72	29
"	9.496	6.373	18	20	-18-		-	-	-	56	27
"	8.469	6.157	16	14	-39-		-	-	-	69	--
"	10.230	6.725	32	24	-38-		-	-	-	94	--
"	9.564	6.439	20	23	-41-		-	-	-	84	--
"	11.134	7.854	16	24	-35-		-	-	-	75	--
"	9.645	6.272	31	24	-44-		-	-	-	99	--
5 Days	7.907	5.017	?	?	23	17	9	-	-	?	20
"	9.153	5.064	22	23	19	14	8	-	-	86	25
"	9.419	6.195	21	20	17	20	14	-	-	92	24

X - Dead, ? - Result Not Known, -n- - Two Days Laying

c) Yemen contd.

Age When Killed	Orig Weight (mg)	Final Weight (mg)	Total Number of Eggs Laid Each Day 1	2	3	4	5	6	7	Tot. Eggs Laid	Total No. of Oocytes
7 Dys	10.512	?	26	20	-40-		14	2	X	102	--
"	11.410	?	31	19	-46-		16	14	1	127	--
"	9.530	?	12	35	-37-		15	11	X	110	--
"	10.892	?	30	25	-31-		5	3	X	94	13
"	9.037	4.962	21	13	-20-		6	8	5	73	20
"	11.771	7.289	17	8	-37-		18	18	2	100	16
"	11.608	6.862	38	20	-42-		14	9	7	130	24
"	4.741	?	20	-23-			12	0	X	X	55	--
"	10.935	6.575	22	-43-			21	26	11	7	130	22
"	9.098	?	43	-40-			18	14	6	X	121	--

X - Dead, ? - Result Not Known, -n- - Two Days Laying

Emergence Weights and Fecundity

Campinas		IITA		Yemen	
Emergence Weight (mg)	No. of Eggs Laid	Emergence Weight (mg)	No. of Eggs Laid	Emergence Weight (mg)	No. of Eggs Laid
5.787	112	5.719	86	6.238	73
5.565	103	5.374	114	7.011	124
5.255	102	4.615	49	8.541	142
5.000	89	6.411	131	6.916	107
5.264	111	4.733	94	7.182	108
6.383	120	6.072	104	5.947	94
4.639	93	4.625	101	9.366	128
3.888	74	6.417	52	5.837	97
5.446	87	3.488	73	7.510	127
5.469	105	5.890	126	5.769	81
6.195	110	4.842	88	7.173	102
3.967	81	6.481	112	6.188	86
3.757	81	6.192	108	6.145	97
4.420	96	5.266	111	8.124	112
5.923	103	5.098	81	8.006	100
4.138	85	7.227	142	7.599	127
4.314	93	6.014	119	6.969	118
6.953	124	5.747	105	7.026	84
4.313	94	6.327	116	8.233	94
3.603	67	6.497	91	7.117	123
2.941	51	6.376	92	7.497	114
4.789	66	5.222	120	6.647	109
4.419	83	6.965	121	6.473	96
3.466	68	3.716	57	6.277	65
5.148	95	4.324	56	6.059	103
3.963	79	4.899	99	8.137	119
5.631	107	3.252	75	9.516	115
4.763	98	4.204	55	6.626	93
3.580	69			8.768	120

6 Day and Lifetime Egg Laying

Egg Laying Period		
0-6 Days	7-Death	Lifetime
94	8	71
101	3	103
86	0	71
91	2	85
64	1	85
111	1	50
62	0	62
79	2	20
91	2	96
60	1	92
105	2	96
49	0	104
79	3	88
70	2	77
87	4	81
64	0	121
89	6	78
73	1	76
74	0	
67	0	

Effect of Reducing the Number of Oviposition Sites

a) Campinas

2 Beans				Control			
Eggs Laid	Eggs Produced	Eggs Unhatched	No. of Oocytes	Eggs Laid	Eggs Produced	Eggs Unhatched	No. of Oocytes
6 HOURS							
9	13	1	36	0	2	-	35
16	19	2	36	9	10	1	35
13	15	0	38	6	7	1	41
22	22	3	38	6	7	1	34
3	3	0	39	6	6	1	42
0	1	-	13	4	6	0	37
20	25	1	39	0	0	-	39
6	9	0	39	0	0	-	?
6	9	0	36				
2 DAYS							
27	39	2	47	32	34	0	33
20	26	1	30	35	35	0	28
30	41	3	33	34	35	0	37
27	28	2	43	27	31	1	38
21	23	0	36	33	35	1	33
21	24	2	34	38	39	8	39
35	36	2	38	27	27	1	41
20	33	1	37	33	37	3	36
24	28	0	31				
5 DAYS							
52	55	2	17	71	73	5	14
59	65	4	22	83	86	9	26
59	63	5	27	64	66	4	27
73	77	5	26	75	77	2	27
46	61	1	21	87	87	16	32
48	62	3	27	39	39	2	15
44	45	0	21	60	62	3	21
58	67	8	18	37	40	3	15
				70	70	0	19
				81	83	7	28

a) Campinas contd.

2 Beans				Control			
Eggs Laid	Eggs Produced	Eggs Unhatched	No. of Oocytes	Eggs Laid	Eggs Produced	Eggs Unhatched	No. of Oocytes
6 DAYS							
24	29	3	14	76	78	2	14
66	79	3	22	86	87	7	21
57	61	5	17	79	79	2	17
64	68	3	16	76	82	4	13
68	74	11	19	81	86	6	21
71	76	8	14	85	88	2	18
72	75	3	19	67	70	13	12
56	62	1	14	86	88	11	22
73	78	10	16	97	98	2	22
84	91	13	?				

b) IITA

2 Beans				Control			
Eggs Laid	Eggs Produced	Eggs Unhatched	No. of Oocytes	Eggs Laid	Eggs Produced	Eggs Unhatched	No. of Oocytes
6 HOURS							
0	2	-	41	0	7	-	38
0	9	-	40	7	12	0	40
5	11	0	40	0	0	-	37
10	25	1	32	0	0	-	29
0	8	-	34	6	8	1	28
3	4	2	49	5	9	5	35
6	9	0	39	0	5	-	34
0	0	-	30	12	16	3	43
0	0	-	36	4	6	1	29
0	0	-	33	0	0	-	27
2 DAYS							
30	39	0	35	38	47	5	28
25	33	1	37	18	24	3	24
12	18	0	36	38	42	0	31
23	33	7	41	40	47	3	30
27	39	3	36	26	33	0	27
26	40	3	28	39	45	1	31
26	37	2	31	18	22	3	19
24	41	11	35	19	29	1	33
20	27	7	29	25	32	5	31
38	46	2	29	41	49	3	37
6 DAYS							
73	78	6	16	92	101	14	29
88	91	5	22	94	96	10	19
83	87	5	18	85	85	15	11
104	107	12	24	86	91	7	18
93	97	4	22	65	69	10	16
84	94	11	14	73	75	4	22
80	82	16	23	89	93	14	13
66	70	3	9	89	94	14	20
80	85	7	18	111	116	8	20
				104	111	32	20

c) Yemen

2 Beans				Control			
Eggs Laid	Eggs Produced	Eggs Unhatched	No. of Oocytes	Eggs Laid	Eggs Produced	Eggs Unhatched	No. of Oocytes
6 HOURS							
10	15	0	41	5	6	1	50
2	13	0	50	5	6	2	37
1	7	0	40	1	11	0	36
0	10	-	37	34	37	1	19
0	9	-	43	2	27	0	34
9	9	1	38	15	16	0	43
13	19	0	36	3	5	0	42
15	24	1	46	11	12	2	38
11	14	0	42	5	7	0	41
7	12	2	36	15	17	0	37
2 DAYS							
24	45	3	42	52	55	3	35
31	52	2	42	41	46	1	42
41	55	1	37	41	50	16	35
24	56	1	31	50	57	1	41
16	42	1	40	34	37	2	37
34	45	0	35	50	59	1	44
27	49	1	37	41	46	14	43
46	59	2	31	48	49	2	37
14	31	0	43	49	51	2	41
26	50	1	32	46	49	2	35
6 DAYS							
84	92	5	15	117	119	5	21
44	46	3	11	101	101	5	27
30	53	5	27	96	101	3	17
43	58	3	19	111	111	3	21
54	60	3	19	116	132	5	16
84	88	6	13	134	138	5	25
70	77	3	18	107	107	6	16
86	92	7	19	109	113	18	22
				111	117	0	26

Eggs per Cowpea	Distribution of Eggs Number of Cowpeas								Total No. of Eggs Laid	Total Unhatched
	0	1	2	3	4	5	6	7	8		
a) Control											
	0	6	13	11	5	3	2	0	0	112	2
	1	9	12	9	7	3	0	0	0	103	2
	1	10	10	8	8	2	1	0	0	102	2
	2	12	12	5	4	2	2	0	0	89	1
	7	5	8	9	2	2	5	1	1	111	4
	2	5	8	9	10	5	0	1	0	120	3
	6	8	8	8	6	1	3	0	0	93	0
	8	11	12	4	4	1	1	0	0	74	1
	3	9	15	8	3	1	0	1	0	87	1
	3	4	14	9	6	2	2	0	0	105	2
	0	6	13	11	5	5	0	0	0	110	0
	7	7	16	5	4	1	1	0	0	81	0
	8	10	10	5	5	2	1	0	0	81	2
	0	8	16	9	6	1	0	0	0	96	4
	3	5	13	8	8	2	1	0	0	103	4
	9	8	9	8	5	0	1	0	0	85	19
	2	9	13	8	7	0	1	0	0	93	2
	2	5	11	7	3	8	3	1	0	124	13
	6	6	9	10	7	1	0	1	0	94	1
	7	14	10	7	1	0	1	0	0	67	0
	9	15	13	2	1	0	0	0	0	51	1
	8	11	11	7	3	0	0	0	0	66	2
	4	13	10	8	4	2	0	0	0	83	1
	11	7	11	7	2	1	0	0	0	68	7
	3	7	10	13	6	1	0	0	0	95	35
	6	11	10	7	3	3	2	0	0	79	1
	2	8	12	8	6	3	2	0	0	107	3
	5	9	10	4	5	5	2	0	0	98	2
	8	11	8	10	3	0	0	0	0	69	1

Eggs per Cowpea	Distribution of Eggs Number of Cowpeas									Total No. of Eggs Laid	Total Unhatched
	0	1	2	3	4	5	6	7	8		
b) Suppressed After Mating											
	5	15	9	10	1	0	0	0	0	67	2
	14	14	8	4	0	0	0	0	0	42	7
	12	12	11	2	1	1	1	0	0	55	4
	21	15	2	2	0	0	0	0	0	25	9
	14	11	6	6	3	0	0	0	0	53	3
	3	17	15	4	2	0	0	0	0	67	2
	9	14	9	4	2	1	1	0	0	63	4
	18	9	7	3	1	1	0	0	0	41	3
	4	15	12	6	3	2	0	0	0	69	0
	7	15	9	8	0	1	0	0	0	62	0
	7	11	13	6	2	1	0	0	0	68	0
	10	13	10	5	0	2	0	0	0	58	2
	2	17	7	8	6	0	0	0	0	79	0
	5	12	9	9	3	2	0	0	0	79	2
	11	13	6	6	3	1	0	0	0	60	1
	12	19	10	0	0	0	0	0	0	39	5
c) Suppressed Before Mating											
	4	11	9	12	4	0	0	0	0	101	0
	7	9	8	10	1	3	0	1	0	81	3
	8	17	7	5	0	1	1	0	0	57	3
	14	15	8	2	1	0	0	0	0	41	10
	1	15	18	5	1	0	0	0	0	70	2
	4	7	11	7	7	1	2	1	0	102	2
	4	10	10	9	4	2	1	0	0	89	1
	3	8	10	18	1	0	0	0	0	86	2
	7	13	12	6	1	0	0	0	0	59	8
	0	8	16	9	6	0	0	0	0	91	1
	10	12	10	4	2	1	1	0	0	63	0
	3	9	15	8	3	1	1	0	0	86	12

Egg Laying on Plain Glass Beads and Cowpeas in Different Arrangements

Eggs per Cowpea - 0	Distribution of Eggs Number of Cowpeas 1	2	3	4	5	6	7	8	Total No. of Eggs Laid	Total Unhatched
a) Cowpeas in Glass Tubes										
5	8	12	5	6	3	0	1	0	93	8
7	8	14	7	4	0	0	0	0	73	4
1	10	17	9	2	1	0	0	0	84	2
8	8	10	9	4	1	0	0	0	78	8
12	10	8	7	1	2	0	0	0	56	6
5	7	11	9	4	3	0	0	1	95	5
3	6	9	10	8	4	0	2	0	120	10
12	8	6	5	6	0	3	0	0	77	4
1	12	12	7	6	1	1	0	0	92	3
2	10	10	7	8	3	0	0	0	98	7
b) Cowpeas in Petri Dishes										
4	11	10	9	5	1	0	0	0	83	5
13	8	11	6	0	1	0	1	0	60	0
2	12	9	10	4	2	1	0	0	94	4
2	7	10	9	8	4	0	0	0	106	3
2	12	14	9	2	1	0	0	0	80	1
2	8	12	9	4	3	1	0	0	96	2
6	13	8	9	3	1	0	0	0	75	1
8	14	8	5	1	3	1	0	0	70	1
11	11	6	7	3	1	1	0	0	67	0
1	11	14	10	4	0	0	0	0	85	2
c) Plain Glass Beads in Tubes										
19	15	5	1	0	0	0	0	0	28	-
11	12	5	7	0	0	0	0	0	63	-
26	11	3	0	0	0	0	0	0	17	-
23	4	7	2	2	1	0	1	0	44	-
30	5	2	2	0	0	0	0	1	23	-
38	2	0	0	0	0	0	0	0	2	-
36	0	3	0	0	0	1	0	0	12	-
17	10	7	2	3	0	1	0	0	48	-
15	14	2	1	4	0	1	0	1	51	-
20	9	4	5	1	0	0	0	1	44	-

Eggs per Cowpea - 0	Distribution of Eggs Number of Cowpeas 1	2	3	4	5	6	7	8	Total No. of Eggs Laid	Total Unhatched
d) Plain Glass Beads in Petri Dishes										
39	1	0	0	0	0	0	0	0	1	-
37	3	0	0	0	0	0	0	0	3	-
31	4	2	0	1	(1x16 + 1x17)				45	-
39	1	0	0	0	0	0	0	0	1	-
35	6	0	0	0	0	0	0	0	6	-
38	3	0	0	0	0	0	0	0	3	-
28	10	3	0	0	0	0	0	0	16	-
40	0	0	0	0	0	0	0	0	0	-
38	3	1	0	0	0	0	0	0	5	-
37	3	0	0	0	0	0	0	0	3	-

Egg Laying on Glass Beads Coated with Cowpea Extract

Eggs per Cowpea - 0	Distribution of Eggs Number of Cowpeas 1	2	3	4	5	6	7	8	9	Total No. of Eggs Laid	Total Unhatched
a) Ordinary Cowpeas											
8	7	8	8	4	4	0	0	1	0	91	4
2	11	11	8	4	2	2	0	0	0	95	1
3	10	16	10	3	0	0	0	0	0	84	11
6	10	6	9	4	1	2	2	0	0	96	7
4	9	15	8	2	1	1	1	0	0	89	9
5	9	7	7	6	2	1	1	0	0	91	9
4	6	12	9	7	2	1	0	0	0	101	3
8	9	13	4	4	2	0	0	0	0	73	4
8	11	6	7	6	2	0	0	0	0	78	2
b) Glass Beads + Extract											
1	5	21	9	4	1	0	0	0	0	95	-
2	6	13	11	6	1	1	0	0	0	100	-
3	11	9	9	5	2	1	0	0	0	94	-
4	17	9	8	2	0	0	0	0	0	67	-
4	23	9	3	1	0	0	0	0	0	54	-
4	15	16	5	0	0	0	0	0	0	61	-
4	11	10	8	4	0	0	0	0	0	71	(+23 loose)
0	5	14	14	5	2	0	0	0	0	105	-
0	15	13	6	4	0	0	0	0	0	75	-
3	11	15	5	4	1	0	0	0	0	77	-
c) Plain Glass Beads (Control)											
20	12	4	2	1	0	0	0	0	0	30	-
15	14	2	4	1	2	1	0	0	0	50	-
25	12	2	0	0	0	0	0	0	0	16	-
35	3	1	0	0	0	0	0	0	0	5	-
9	11	7	8	2	2	0	0	0	0	67	-
11	19	6	3	1	0	0	0	0	0	44	-
20	11	7	1	0	0	0	0	0	0	28	-
19	14	3	2	1	0	0	0	0	0	30	-
3	8	14	8	1	4	1	0	0	0	90	-
14	8	8	5	2	1	0	0	0	0	52	-

Eggs per Cowpea - 0	Distribution of Eggs Number of Cowpeas 1	2	3	4	5	6	7	8	9	Total No. of Eggs Laid	Total Unhatched
d) Extracted Cowpeas											
8	10	8	9	0	0	1	1	1	1	83	12
13	7	6	5	3	3	3	0	0	0	79	5
6	14	9	3	3	3	1	0	0	1	83	15
3	9	12	7	5	3	0	1	0	0	96	3
6	20	6	3	2	0	0	0	0	0	49	12
4	11	13	6	3	1	2	0	0	0	84	6
9	15	6	4	0	2	3	0	0	1	86	5
4	13	12	9	2	0	0	0	0	0	72	56
11	9	8	5	2	2	1	1	0	0	71	5

Time Required to Obtain an Effective Cowpea Extract

Eggs Laid	Eggs Produced	Eggs Laid	Eggs Produced	Eggs Laid	Eggs Produced
Control		10 Min Extract		1 Hour Extract	
32	60	9	17	64	65
26	44	79	81	96	97
67	74	100	104	94	96
65	73	65	66	57	58
47	48	83	84	89	89
25	39	88	94	93	94
31	53	74	75	88	90
34	59	89	91	81	84
11	50	104	105	45	76
29	36	82	84		
6 Hour Extract		24 Hour Extract		Cowpeas	
0	20	14	26	96	98
14	41	74	76	94	98
62	63	48	48	83	85
10	32	27	49	92	94
10	36	77	82	89	93
14	36	60	61	83	85
23	38	11	36	111	112
		24	41	110	111
		2	18	86	88
		69	71	109	109

Egg Laying on Different Concentrations of a 10 Minute Extract

Eggs Laid	Eggs Produced	Eggs Laid	Eggs Produced	Eggs Laid	Eggs Produced
Control		Normal Conc.		2X Normal Conc.	
62	80	99	100	63	64
54	66	81	83	77	86
3	48	89	92	96	97
14	44	82	87	101	104
21	53	97	98	53	73
17	45	105	106	56	60
9	19	100	103	87	93
36	57	79	86	81	82
		101	104	68	70
				98	99
4X Normal Conc.		8X Normal Conc.		Cowpeas	
76	80	94	97	96	99
92	94	101	104	77	77
96	96	88	97	96	102
81	83	114	114	80	94
78	79	78	78	80	83
43	60	72	73	82	82
49	66	68	69	108	114
100	103	82	84	83	84
78	79	82	83	92	95
30	55				

Egg Laying on Different Concentrations of a 24 Hour Extract

Eggs Laid	Eggs Produced	Eggs Laid	Eggs Produced	Eggs Laid	Eggs Produced
Control		0.1X Normal Conc.		0.25X Normal Conc.	
59	64	67	75	69	71
84	85	60	62	100	103
87	87	97	98	93	95
21	41	105	106	89	92
40	45	90	93	94	95
41	49	99	99	93	98
15	32	86	87	93	97
53	60	97	98	92	94
57	59	45	47	106	107
		83	84	77	79
0.5X Normal Conc.		Normal Conc.		Cowpeas	
85	86	84	84	114	115
90	96	86	94	77	79
66	68	105	105	94	94
102	104	121	121	79	79
72	74	84	85	115	116
106	107	104	107	84	84
103	104	105	104	85	85
79	82	78	82	84	86
73	?	81	83	76	77

Eggs per Cowpea - 0	Distribution of Eggs 1	2	3	4	5	6	7	8	Total No. of Eggs Laid	Total Unhatched
	Number of Cowpeas									
a) Mated Once										
14	11	12	1	2	0	0	0	0	46	0
5	11	10	9	2	3	0	0	0	81	9
11	16	9	2	1	1	0	0	0	49	5
0	8	9	15	4	2	0	2	0	111	6
7	8	11	8	3	3	0	0	0	81	4
0	3	8	12	7	8	2	0	0	135	5
4	7	7	9	9	2	1	0	1	108	6
7	12	7	10	1	2	1	0	0	76	7
6	4	13	9	5	1	1	1	0	95	12
2	8	13	9	2	5	0	1	0	101	4
3	6	11	12	5	2	1	0	0	106	16
21	15	1	3	0	0	0	0	0	26	4
4	10	8	12	2	2	2	0	0	92	1
4	11	8	8	6	3	0	0	0	90	4
2	7	8	11	7	3	1	0	1	113	4
5	10	10	9	2	1	1	1	1	91	5
1	3	8	11	11	6	0	0	0	126	9
2	2	14	9	6	4	2	1	0	120	4
b) Remated										
1	10	11	7	8	1	2	0	0	102	10
3	2	10	6	13	3	3	0	0	125	9
6	12	5	8	3	1	4	0	1	95	8
8	8	13	7	2	1	1	0	0	74	6
5	12	9	11	2	1	0	0	0	76	5
5	12	10	8	4	1	0	0	0	77	9
2	10	12	8	4	1	3	0	0	97	2
3	6	4	14	9	4	0	0	0	112	2
9	6	11	7	4	2	1	0	0	81	6
7	15	3	11	6	2	0	1	0	95	7
4	13	9	7	3	2	1	1	0	87	2
0	9	10	7	11	0	2	1	0	113	9
3	6	18	9	2	2	0	0	0	87	7
1	6	7	12	5	4	5	0	0	126	3
1	9	9	5	7	7	1	1	0	118	8
1	9	6	7	10	3	3	1	0	122	4

The effect of different numbers of males

Eggs per Cowpea - 0	Distribution of Eggs Number of Cowpeas 1	2	3	4	5	6	7	8	9	Total No. of Eggs Laid	Total Unhatched
a) No male											
5	10	11	10	2	2	0	0	0	0	80	4
2	7	9	10	8	4	0	0	0	0	107	9
6	13	11	7	2	1	0	0	0	0	69	5
14	11	7	7	1	0	0	0	0	0	50	3
4	11	15	5	4	0	0	1	0	0	79	6
2	6	13	10	4	3	1	1	0	0	106	6
7	7	11	7	3	4	0	0	0	1	91	6
4	8	7	8	6	4	2	0	1	0	110	7
6	10	12	7	3	3	0	0	0	0	82	4
6	16	7	4	5	2	0	0	0	0	72	15
3	6	5	15	5	4	2	0	0	0	113	17
4	9	9	11	7	0	0	0	0	0	88	7
1	6	12	11	7	2	1	0	0	0	107	11
1	9	11	12	5	1	0	1	0	0	99	2
0	7	13	12	7	1	0	0	0	0	102	4
3	7	10	10	5	5	0	0	0	0	102	13
3	7	10	9	8	2	0	1	0	0	103	7
3	7	6	10	7	5	1	1	0	0	115	6
6	10	8	6	4	4	2	0	0	0	92	3
b) 1 male											
16	10	7	5	2	0	0	0	0	0	47	3
3	15	8	5	4	2	2	1	0	0	89	3
7	14	11	3	3	1	1	0	0	0	68	6
4	8	7	13	5	2	1	0	0	0	97	4
9	10	10	5	4	1	1	0	0	0	72	5
9	7	10	10	3	0	0	1	0	0	76	8
3	10	12	6	5	2	1	0	0	0	88	6
1	6	6	9	5	6	1	0	0	0	101	19
3	8	10	9	4	3	3	0	0	0	104	15
7	10	8	7	6	0	1	1	0	0	84	16
1	7	9	8	9	4	2	0	0	0	117	4
6	12	8	6	8	1	0	0	0	0	83	6
6	6	13	8	4	2	1	0	0	0	88	5
3	4	9	11	8	4	1	0	0	0	113	9
1	3	12	9	10	2	3	0	0	0	122	7
3	5	9	12	6	5	0	0	0	0	108	5
5	8	9	12	4	1	1	0	0	0	89	2
4	9	10	6	2	8	1	0	0	0	101	21
7	7	8	9	6	3	0	0	0	0	89	9

Eggs per Cowpea - 0	Distribution of Eggs Number of Cowpeas 1	2	3	4	5	6	7	8	9	Total No. of Eggs Laid	Total Unhatched
c) 5 males											
9	6	9	5	7	3	1	0	0	0	88	7
3	10	6	9	2	1	3	0	1	0	88	7
10	6	11	7	2	1	2	1	0	0	81	14
6	20	7	2	4	1	0	0	0	0	61	8
8	13	10	7	1	1	0	0	0	0	63	2
2	13	10	8	4	1	1	1	0	0	91	14
2	6	15	12	2	2	1	0	0	0	96	6
6	12	9	4	7	0	1	1	0	0	83	14
9	13	8	6	3	1	0	0	0	0	72	5
0	8	16	6	7	1	2	0	0	0	103	5
1	9	9	12	4	5	0	0	0	0	104	8
3	6	11	12	7	1	0	0	0	0	97	7
7	7	5	10	9	1	1	0	0	0	94	8
8	4	10	9	5	1	2	0	1	0	96	5
0	16	12	7	1	1	2	1	0	0	89	12
3	10	10	7	4	5	1	0	0	0	98	13
6	5	12	10	3	3	1	0	0	0	92	13
d) 10 males											
5	5	9	10	7	0	4	0	0	0	105	9
1	6	12	10	6	3	1	1	0	0	112	10
10	10	8	7	2	3	0	0	0	0	70	6
4	12	9	11	2	0	1	1	0	0	84	18
9	7	9	3	7	1	2	0	1	1	96	6
4	10	9	8	6	0	2	1	0	0	95	12
11	18	6	4	1	0	0	0	0	0	46	3
3	10	9	8	9	1	0	0	0	0	93	7
5	12	9	8	2	1	3	0	0	0	85	7
4	7	12	5	7	5	0	0	0	0	99	2
2	13	6	8	5	3	2	0	0	1	105	17
5	9	15	3	3	2	2	1	0	0	89	5
7	9	9	7	3	3	0	0	1	0	83	7
8	11	7	4	3	5	2	0	0	0	86	10
0	5	13	13	8	1	0	0	0	0	107	13
1	5	4	6	11	5	5	2	1	0	152	17
5	9	7	9	7	2	1	0	0	0	90	10

No choice effect of egg marker over 6 days

Eggs per Cowpea - 0	Distribution of Eggs Number of Cowpeas 1	2	3	4	5	6	7	8	Total No. of Eggs Laid	Total Unhatched
a) Ordinary cowpeas										
4	14	10	7	0	3	2	0	0	82	23
3	13	10	5	6	3	0	0	0	87	12
2	8	10	11	6	2	1	0	0	101	9
2	10	15	7	3	1	2	0	0	90	7
2	9	13	8	5	1	1	1	0	97	12
4	9	8	8	9	2	0	0	0	95	4
6	7	9	5	5	5	1	2	0	105	14
5	11	12	5	3	4	0	0	0	82	12
5	6	12	6	5	5	1	0	0	105	6
b) Control cowpeas										
3	6	14	8	8	1	0	0	0	95	28
3	10	11	6	2	4	2	2	0	104	15
1	7	16	10	5	1	0	0	0	94	4
4	8	13	10	3	2	0	0	0	86	9
3	5	14	5	9	3	1	0	0	105	17
2	8	17	7	4	2	0	0	0	89	34
5	17	13	2	1	1	1	0	0	64	6
5	13	16	5	1	0	0	0	0	64	39
3	7	16	10	3	0	1	0	0	87	11
1	5	18	11	5	0	0	0	0	94	10
c) Marked cowpeas										
7	7	11	7	5	3	0	0	0	85	10
4	10	14	11	1	1	0	0	1	88	9
9	10	7	4	6	1	2	0	1	85	7
4	10	11	6	7	2	0	0	0	88	12
6	10	7	4	5	3	3	2	0	103	6
8	6	11	6	2	3	3	1	0	94	11
7	8	5	8	8	3	0	1	0	96	12
7	8	12	6	6	0	0	1	0	81	10
1	7	14	5	3	1	3	0	1	93	8
4	9	10	7	7	3	0	0	0	93	8

No choice effect of egg marker over 12, 24 and 48 hours

Eggs per Cowpea - 0	Distribution of Eggs Number of Cowpeas 1	2	3	4	5	6	7	8	Total No. of Eggs Laid	Total Unhatched
a) 12 Hours										
Control cowpeas										
7	1	2	0	0	0	0	0	0	5	0
3	3	3	1	0	0	0	0	0	12	1
0	0	0	0	0	0	0	0	0	0	-
1	3	4	1	0	0	1	0	0	20	0
3	5	2	0	0	0	0	0	0	9	1
3	3	1	2	0	1	0	0	0	16	1
2	3	4	0	1	0	0	0	0	15	2
3	1	4	1	0	1	0	0	0	17	1
6	3	1	0	0	0	0	0	0	5	0
2	4	2	0	1	1	0	0	0	17	0
Marked cowpeas										
2	3	2	1	0	1	1	0	0	21	6
0	3	4	1	1	0	0	(1	x11)	29	1
2	3	2	2	0	0	1	0	0	19	0
3	1	3	3	0	0	0	0	0	16	2
4	4	2	0	0	0	0	0	0	8	1
3	2	3	1	0	1	0	0	0	16	2
6	2	2	0	0	0	0	0	0	6	0
4	2	3	0	0	0	1	0	0	14	0
4	2	3	0	1	0	0	0	0	12	3
5	3	1	0	0	1	0	0	0	10	1
b) 24 Hours										
Control cowpeas										
0	0	4	1	5	0	1	0	0	39	2
1	0	6	1	1	0	1	0	0	25	3
0	4	2	2	1	1	0	0	0	23	0
0	2	5	2	1	0	0	0	0	22	3
2	1	3	2	1	0	1	0	0	23	2
2	2	3	3	0	0	0	0	0	17	1
0	2	1	5	2	0	0	0	0	27	2
0	3	2	2	2	1	0	0	0	26	2
2	1	3	2	2	0	0	0	0	21	2
2	2	3	3	0	0	0	0	0	17	1

Eggs per Cowpea - 0	Distribution of Eggs Number of Cowpeas 1	2	3	4	5	6	7	8	9	Total No. of Eggs Laid	Total Unhatched
b) 24 Hours (contd.)											
Marked cowpeas											
1	2	1	3	2	0	1	0	0	0	27	0
2	2	4	0	0	2	0	0	0	0	20	1
1	2	2	3	1	1	0	0	0	0	24	1
2	1	3	0	2	2	0	0	0	0	25	3
10	0	0	0	0	0	0	0	0	0	0	-
1	5	0	2	2	0	0	0	0	0	19	1
0	1	3	3	1	0	1	0	1	0	34	1
2	1	2	2	2	1	0	0	0	0	24	1
2	4	3	1	0	0	0	0	0	0	13	2
3	2	0	1	1	1	1	1	0	0	27	5
c) 48 Hours											
Control cowpeas											
0	1	1	0	0	2	4	0	8	0	53	2
0	0	0	0	4	2	2	1	1	0	53	4
0	0	2	0	2	2	1	0	2	0	53	5
0	0	1	3	4	1	1	1	0	0	45	3
0	1	0	0	4	1	2	1	0	1	50	3
0	0	0	2	2	2	3	0	1	0	50	3
0	0	2	2	1	2	2	1	0	0	43	4
0	0	0	1	5	2	1	0	0	1	48	6
0	0	0	0	5	3	0	1	0	1	51	2
0	1	3	2	2	2	0	0	0	0	37	4
Marked cowpeas											
1	1	2	1	0	0	2	3	0	0	41	5
0	0	2	2	2	0	1	2	(1 x11)		49	3
0	0	0	4	2	1	0	2	0	1	48	6
1	0	1	1	1	2	2	0	1	(1 x10)	49	3
0	0	2	1	3	2	1	1	0	0	42	2
0	0	1	2	0	2	1	0	2	0	42	5
1	2	2	2	0	0	1	2	0	0	32	4
1	1	0	0	1	2	2	1	1	1	51	2
0	0	0	1	2	3	2	0	1	0	46	6
0	2	0	2	3	2	0	1	0	0	37	3

Eggs laid on marked and control cowpeas in first choice chamber experiment.

Number of eggs laid in each section of the choice chamber.	
Marked	Control
6	12
9	11
2	8
8	14
1	15
2	12
7	11
0	12
4	15
6	10
11	3
3	16
1	9
3	8
3	9
6	8
3	15
1	12

Eggs laid on marked and control cowpeas when they were intermingled

Number of eggs laid in each section of the choice chamber.	
Marked	Control
2	17
3	22
2	19
4	24
4	25
4	19
3	14
2	10
7	14
1	19
4	23
3	25
4	21
6	27
4	24
0	27
8	20
4	24
5	25
5	20

Eggs laid on marked and control cowpeas when different solvents were used to collect the marker.

Number of eggs laid in each section of the choice chamber.	
Marked	Control
a) Dichloromethane	
13	12
7	14
10	12
12	15
12	14
14	14
9	15
7	18
22	24
11	14
13	19
8	12
6	9
7	9
14	13
6	8
13	14
10	13
5	14
b) Petroleum ether	
10	21
7	12
10	21
10	16
11	8
7	20
7	14
6	12
12	22
6	14
9	13
7	16
8	10
7	18
5	8
3	13
7	11
13	10

	Number of eggs laid in each section of the choice chamber.	
	Marked	Control
c) Acetone		
	5	21
	7	11
	14	31
	2	3
	10	21
	5	15
	5	15
	3	11
	8	13
	5	12
	6	6
	6	18
	5	17
	10	14
	6	8
	10	13
	5	16
	8	17
	10	13
d) Water		
	15	24
	9	30
	15	10
	5	23
	13	15
	9	22
	6	17
	14	17
	10	18
	11	19
	11	24
	10	19
	9	22
	9	21
	5	16
	6	12
	13	16
	11	15
	10	24
	5	17

Eggs laid on marked and control cowpeas at different concentrations of marker.

Number of eggs laid in each section of the choice chamber.	
Marked	Control
a) 4 egg equivalents	
9	18
15	21
8	26
15	11
18	25
5	18
24	16
18	16
11	15
12	16
6	14
10	14
10	11
19	11
14	17
8	15
11	23
13	12
9	16
b) 2 egg equivalents	
13	15
13	28
11	14
13	10
6	17
15	18
7	12
11	12
17	18
16	16
14	13
13	13
12	14
9	18
11	14
12	13
13	6
7	10
9	7

Number of eggs laid in each section of the choice chamber.	
Marked	Control
c) 1 egg equivalent	
18	15
20	18
14	8
16	15
15	20
12	17
13	13
16	13
7	12
15	17
16	22
21	14
11	14
10	13
11	19
11	9
4	10
12	12
15	13
7	11
d) 0.5 egg equivalents	
15	18
14	12
17	17
11	10
7	15
12	14
16	13
19	18
14	15
5	5
7	10
8	10
10	16
10	9
6	1
7	3
11	4
10	8
14	9
14	11

Eggs laid on marked and control cowpeas with different ages of marker.

Number of eggs laid in each section of the choice chamber.	
Marked	Control
a) Fresh marker	
14	12
9	22
13	17
14	13
18	20
13	18
6	11
13	12
11	15
13	14
10	12
0	1
7	20
12	9
12	16
b) 7-day old marker	
15	24
7	20
11	18
10	14
11	13
11	16
13	17
20	16
6	11
16	19
14	15
7	6
8	8
8	16
6	19

Number of eggs laid in each section of the choice chamber.	
Marked	Control
c) 14-day old marker	
18	16
11	12
20	20
13	21
10	21
14	10
12	12
20	25
13	13
17	17
16	8
10	16
2	0
9	18
13	11
d) 30-day old marker	
17	27
18	15
12	20
15	24
9	19
9	17
14	24
14	21
10	13
7	17
12	11
19	17
17	12
4	1
6	12

Eggs laid on marked and control cowpeas with different sources of marker.

Number of eggs laid in each section of the choice chamber.	
Marked	Control
a) Egg laying females	
4	20
6	20
2	6
2	23
0	18
1	35
1	16
5	17
2	12
4	16
3	11
0	19
5	20
2	15
3	13
2	15
5	16
5	13
b) Females artificially prevented from laying eggs	
4	15
2	16
11	12
13	18
6	18
10	14
8	19
11	9
1	12
8	12
2	17
9	10
10	13

Number of eggs laid in each section of the choice chamber.	
Marked	Control

c) Males

8	18
9	6
10	15
14	7
7	9
10	16
4	15
9	10
4	17
10	21
7	15
11	12
14	21
8	6
8	6
6	12
6	15

Strain comparison of eggs laid on marked and control cowpeas.

Number of eggs laid in each section of the choice chamber.	
Marked	Control
a1) Campinas pairs with IITA marker	
8	17
3	14
12	15
7	17
1	12
6	16
5	18
3	15
4	20
6	12
10	12
7	16
5	11
4	16
6	12
11	18
3	9
6	18
0	19
1	8
a2) Campinas pairs with Campinas marker	
10	19
7	18
7	6
11	18
6	17
7	19
10	3
3	21
13	4
3	11
3	4
6	20
3	19
7	13
9	7
3	19
5	16
6	24
2	9

Number of eggs laid in each section of the choice chamber.	
Marked	Control
a3) Campinas pairs with Yemen marker	
9	19
0	10
1	22
5	12
3	20
6	15
9	13
3	14
3	15
3	16
6	15
3	20
0	16
4	16
5	20
2	10
2	20
2	17
4	14
2	13
b1) IITA pairs with IITA marker	
1	29
5	15
3	13
0	4
2	12
4	22
2	6
6	20
4	6
3	1
4	11
0	18
1	10
5	22
4	9
2	11
3	12
10	3
3	11

Number of eggs laid in each section of the choice chamber.	
Marked	Control

b2) IITA pairs with Campinas marker

0	15
3	23
3	11
5	31
5	14
3	11
13	9
4	11
17	6
8	13
19	13
1	23
3	0
0	11
2	11
1	3
1	0
1	9

b3) IITA pairs with Yemen marker

2	11
1	18
11	6
12	27
4	10
0	12
9	13
3	16
3	21
1	9
1	11
1	10
2	6
2	14
1	8
0	7

Number of eggs laid in each section of the choice chamber.	
Marked	Control
c1) Yemen pairs with IITA marker	
8	41
1	35
2	29
11	25
4	29
6	22
10	21
4	27
2	24
2	20
6	26
7	23
2	9
1	43
2	17
4	31
1	28
4	28
4	20
c2) Yemen pairs with Campinas marker	
7	34
5	33
6	28
7	21
0	38
7	16
5	33
5	18
8	20
15	19
4	27
4	29
0	1
7	15
5	20
5	37
5	28
7	33
11	18

Number of eggs laid in each section of the choice chamber.	
Marked	Control

c3) Yemen pairs with Yemen marker

1	28
0	19
3	28
1	30
1	35
2	39
2	22
4	30
0	29
0	32
3	12
0	19
1	16
5	26
3	19
0	31
4	36
2	13
2	39

Number of eggs laid on large and medium-sized glass beads.

Number of eggs laid on each type of glass bead in each Petri dish.	
Large	Medium
12	22
10	27
9	26
12	14
10	32
0	30
2	27
0	20
16	21
10	23
14	20
19	17
13	19
16	18
0	1
16	17
2	18
11	19
14	19

Number of eggs laid on small and medium-sized glass beads.

Number of eggs laid on each type of glass bead in each Petri dish.	
Medium	Small
20	5
18	13
26	11
26	8
17	9
23	13
28	8
19	10
21	1
15	5
22	5
22	7
14	14
15	12
23	9
21	12
24	12
27	2
14	9

Number of eggs laid on small, medium and large glass beads.

Number of eggs laid on each type of glass bead in each Petri dish.		
Small	Medium	Large
1	10	10
1	19	15
12	20	3
4	16	8
7	16	20
8	7	0
6	14	5
0	5	7
1	8	13
0	25	6
16	16	6
9	18	15
9	6	3
8	11	7
11	17	1
6	10	5
5	15	7
12	7	5
4	10	5
8	22	3

Individual weight (mg) of cowpeas
with different egg loads

(Beetle density - 5 pairs)

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0 Eggs per cowpea

242	191	172	293	298	197	219	243	259	274
311	225	303	262	277	197	203	264	330	290
248	179	252	211	207	188	217	277	199	244
247	260	272	243	306	280	254	233	315	298
308	319	332	315	302	366	273	292	296	273
256	271	262	243	260	265	245	283	251	290
268	260	262	230	244	263	222	268	271	263
244	246	262	279	255	234	238	256	251	229
236	212	188	201	208	238	182	190	233	219
186	206	201	251	194	215	224	242	218	169
218	185	197	174	163	159	181	198	178	173
149	169	166	184	162	151	162	150	146	181
150	123	153	147	125	149	148	134	138	106
115	90	137	101	106	75	77	82	74	63
58									

1 Egg per cowpea

305	329	331	315	355	311	303	294	299	319
302	285	284	280	252	285	278	193	250	229
234	220	247	241	201	230	219	209	226	212
200	216	198	208	213	215	182	200	218	158
164	164	161	157	149	120	116	121	99	

2 Eggs per cowpea

361	331	316	248	220	187	214	169	156	169
138	139	140	107	127					

3 Eggs per cowpea

283	237	190

4 Eggs per cowpea

304

Individual weight (mg) of cowpeas with different egg loads

(Beetle density - 10 pairs)

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0 Eggs per cowpea

328	344	330	342	290	335	325	336	337	307
322	332	311	306	307	331	298	301	283	313
275	281	296	310	304	288	283	300	267	273
296	265	284	224	259	265	229	253	239	249
225	250	230	206	235	206	198	207	244	180
220	195	183	206	199	181	212	183	217	191
193	180	204	155	179	163	177	166	151	150
148	138	156	122	103	112	104	91	97	

1 Egg per cowpea

310	343	322	310	324	322	319	322	305	357
296	324	301	296	303	273	273	279	291	275
271	243	269	265	255	258	248	259	283	265
248	267	261	266	224	251	208	216	235	192
224	235	221	233	237	224	205	203	212	210
219	206	199	172	204	168	216	177	178	137
129	78								

2 Eggs per cowpea

284	313	332	338	311	292	262	279	308	295
266	270	283	253	298	256	237	246	243	258
226	264	240	226	180	247	245	236	190	205
207	169	199	179	151	158	150	105		

3 Eggs per cowpea

338	306	287	270	243	255	235	214	201	188
142	85								

4 Eggs per cowpea

238	250	191

5 Eggs per cowpea

174

Individual weight (mg) of cowpeas with different egg loads

(Beetle density - 20 pairs)

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0 Eggs per cowpea

340	250	298	321	265	294	303	263	235	219
189	211	190	197	173	161	173	141	124	134
117	104	130	63						

1 Egg per cowpea

347	368	354	307	318	334	291	315	323	284
259	234	268	297	252	274	252	263	255	252
244	249	214	261	217	247	214	222	206	235
200	187	211	185	178	186	159	182	178	138
131	162	128	74						

2 Eggs per cowpea

348	306	278	283	325	272	300	308	270	319
267	286	238	245	248	264	235	266	270	248
248	246	214	223	241	261	249	229	220	206
210	211	196	181	156	117	69			

3 Eggs per cowpea

324	303	305	315	280	324	272	297	280	292
262	282	197	295	268	229	264	272	250	156
255	242	220	242	198	250	134	214	197	191
133	139	90	73						

4 Eggs per cowpea

335	324	292	335	321	347	315	283	305	261
361	316	288	297	253	287	279	270	266	280
275	302	289	271	224	233	221	218	212	236
173	156	174	148	129	109	77			

5 Eggs per cowpea

367	329	234	268	258	234	238	214	212	185
209	181	181							

6 Eggs per cowpea

288	335	287	325	265	275	221	197	153

7 Eggs per cowpea

311	202

8 Eggs per cowpea

212	186

Individual weight (mg) of cowpeas with different egg loads

(Beetle density - 200 unsexed)

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1 Egg per cowpea

183 147 243

2 Eggs per cowpea

175

3 Eggs per cowpea

252 204 228 136
173 134 120 149

4 Eggs per cowpea

275 259 285 228
203 217 160 166
139 102

5 Eggs per cowpea

272 253 288 277
244 239 223 200
195 192 171 158
197 107 59

6 Eggs per cowpea

281 300 269 310
228 217 176 161
125 113

7 Eggs per cowpea

310 247 214 267 259 221 195 255 204 206
209 195 194 211 183 178

8 Eggs per cowpea

330 314 258 289 261 253 280 231 241 206
234 293 207 228 211 181 176 196 184 153
158 117 112 81 71 83 81 70

9 Eggs per cowpea

303 313 267 275 291 255 287 267 245 245
253 232 212 230 207 212 203 160 91

10 Eggs per cowpea

312 315 283 276 269 290 244 255 261 239
230 210 251 192 208 210 235 187 179 201
162 157 178 158

11 Eggs per cowpea

261 273 277 283 282 269 277 278 222 294
229 242 254 309 237 175 225 220 185 231
210 145 123

Individual weight (mg) of cowpeas
with different egg loads.

(Beetle density - 200 unsexed (Contd.).)

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12 Eggs per cowpea

278 302 273 225
234 251 240 265
239 246 182 190
149 148

13 Eggs per cowpea

325 321 172 215

14 Eggs per cowpea

301 285 292 259
331 258 203 191
208 185 217 210
149

15 Eggs per cowpea

283 290 307 262
250 246 150

16 Eggs per cowpea

263 198

17 Eggs per cowpea

270 199

18 Eggs per cowpea

236

20 Eggs per cowpea

311

Number of eggs laid on cowpeas with 1, 2 and 3 eggs already attached

Number of eggs laid on each type of cowpea in each Petri dish. (Number of eggs already attached)		
1-Egg	2-Eggs	3-Eggs
10	6	7
7	6	13
3	7	13
7	13	12
6	5	6
4	4	0
8	9	7
5	8	9
8	8	10
9	8	12
8	13	11
11	12	8
4	8	6
9	5	11
4	8	11
9	4	6
7	7	7
10	9	10
6	3	1
2	7	6

The eggs on the cowpeas at the start of the experiment are not included in the totals given here.