THE BEHAVIOUR OF GRAIN-INFESTING BEETLES WITH REFERENCE TO THE EFFECTS OF GAMMA-IRRADIATION UPON DEVELOPMENT OF POPULATIONS AND INTRA-SPECIFIC COMMUNICATION.

by

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Summary

Biological studies involving measurement of their innate capacities for increase were completed upon both rice weevil (Sitophilus oryzae) and lesser grain borer (Rhizopertha dominica). The values for r_c and r_m calculated were similar to those obtained by other entomologists using slightly different rearing regimes. Sterilizing doses of irradiation from ^{60}Cobalt were found to be 10 Krads for rice weevil and 12 Krads for lesser grain borer and while their capacities to compete with non-irradiated individuals of their own kind were not impaired, irradiation did greatly reduce longevity of adults of both species. A radioisotope technique developed to monitor dispersal of rice weevil individuals in columns of grain, resulted in strong indications of male attraction to female. Olfactometric studies clearly demonstrated that pheromones are produced by both sexes in this insect, that the chemicals involved may be collected, isolated and bioassayed and should be identifiable and perhaps able to be synthesized.

The use of aggregation of the sexes and sterile insect release as components of a pest management programme in food storages is discussed.

Declaration

The work presented in this thesis is my own unless otherwise acknowledged, and has not previously been published or submitted to any university for the award of any degree.

(Muhammad Zainul Abedin Khan)

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1. GENERAL INTRODUCTION

Insects constitute one of the greatest hazards to the safe storage of grain (Cotton and Ashby, 1952). A survey carried out in 1947 by the Food and Agricultural Organization of the United Nations, estimated annual losses of about 8% sustained in cereal storage and caused by insects. This excluded losses during farm storage. If these losses were included, total losses would amount to 10 percent of the world's annual production of cereals (Munro 1966).

Attempts have been made to assess the value of food destroyed by insects in specific countries. Fairfield estimated, that in 1943, the total loss of foodstuffs in U.S.A., including damage during growth and storage, approximated 2 billion dollars. Of this she attributed losses of 300 million dollars to pests of stored cereals, including 60 million dollars to the rice weevil alone. In 1951, Bishop revised these estimates, largely because of increases in commodity prices, to 4 billion dollars with stored cereal losses amounting to 600 million dollars (Parkin, 1956). Haeussler (1952) published similar figures giving the amount of grain lost annually to insects as 300 million bushels valued at 500 million dollars based on 1951 prices.

The grain weevils are among the world's most destructive pests of stored food (Howe, 1952) and Sitophilus oryzae (L.), the rice weevil, has been rated that most destructive species (Cotton, 1950). Supposedly a native of India, this weevil is now distributed all over the world and attacks a variety of food such as rice, wheat, corn, macaroni, oats, barley, sorghum, Kaffir seed, buck-wheat, and other grain and grain products. It also attacks corn in the field in the U.S.A., south of about 39^{o}N latitude and especially following damage by the corn earworm, Heliothis zea (Metcalf, Flint and Metcalf, 1962).

The lesser grain borer, Rhizopertha dominica (Fabricius), another important pest of stored grain, also believed to be indigenous to India and now cosmopolitan. In addition to stored grain, the lesser grain borer has been recorded from edible bulbs and bread made from them, wild prairie turnips, ships biscuits, malted barley and a wide variety of other starchy products (Potter, 1935).

The control of stored grain pests has so far been achieved mainly with contact insecticides and fumigants, but proven instances of the development of resistance to chemicals used in both of these control systems are increasing. Ten species of stored product pests, including both the rice weevil and the lesser grain borer, have become resistant to some or all of the contact insecticides and acaricides commonly used in control programmes in many countries of the world (Freeman, 1974).

The use of insecticides may have other serious drawbacks, such as environmental pollution, public health hazards, reduction of beneficial and non-target organisms and the chain of imbalances this effect may generate among plant and animal biosystems (Carson, 1962).

In addition, the rapidly increasing costs of chemical control programmes, in part due to rapid price rises and apparent shortages of petroleum products in recent times, is becoming another limitation against such controls; and costs are likely to increase with time, especially in the developing nations. Shortages of insecticides have been reported in some developing countries like India. Phillipines and Nicaragua in 1974 (Cavin, 1975). These factors pose a serious threat to the future of chemical controls for insect pests including stored product pests, especially in countries where food production, storage and distribution are important to the ability of nations to feed and clothe their increasing human populations.

Scientists have widely accepted the challenges innate to food

protection by continuing to explore ways and means of finding and testing effective and safer alternatives for controlling pest populations. Among such technological developments arising from novel research programmes are controls based upon sterile insect release, chemosterilants, and the manipulation of behaviour and development by using specific pheromones, attractants, repellants, antifeedants and hormones.

Among these systems, the use of sterile insect releases (Knipling, 1955, 1959) to combat insect pests has proven effective under particular conditions. The concept is simply the application of intra-specific destruction by introduction of induced sterility into natural populations. This is effected by releases of the artificially sterilized individuals in particular numerical ratios with the wild males in the populations of concern. The ratio of sterile to fertile males may vary with the species.

Since the first application of this method and the successful eradication of the screw worm fly from the Island of Curacao (Baumhover et al., 1955; Lindquist, 1955), several other insect pests have been controlled or eradicated in different parts of the world (LaChance, 1974).

The sterile insect release method, integrated with other control systems, could be applied more usefully for the control of many more insect pests than the sterile insect method alone. The possibilities of such integrated controls have been suggested and discussed in detail (Knipling, 1964, 1968; LaChance et al., 1967, LaChance, 1974). A few examples of the success of the sterile insect release method integrated with other methods to suppress or eradicate pest populations are: the eradication of the melon fly, Dacus cucurbitae Coquillet from Rota Island (Steiner et al., 1965); eradication of the Oriental fruit fly, Dacus dorsalis Hendel from Mariana Island (Steiner et al., 1970); suppression of the Mediterranean fruit fly, Ceratitis capitata (Wiedemann) in Nicaragua (Rhode et al.,

1971) and the suppression of the olive fly, Dacus oleae (Gmel.) in Kassandra, Northern Greece (Economopoulos et al., 1977).

The sterile insect release technique has distinct advantages over chemical insecticides. Induced sterility is species-specific, does not leave residues and is non-polluting. If successfully and continually applied, it can lead to total eradication of a pest.

Theoretically, induced sterility is applicable to all sexually reproducing species, but its application requires a sound knowledge of the biology, ecology, behaviour and population dynamics of the candidate species (Knipling, 1968). Its feasibility depends upon a number of innate factors which may vary according to the species considered. Some of these requirements have been listed (Knipling, 1955, 1968). The sterilization procedures and their effects upon mating competitiveness, longevity and dispersive behaviour of the sterile males, especially at low density, are of considerable importance to the achievement of suppression or eradication of the pest populations.

Sterilization of insects may be achieved in a number of ways such as by radiation sterilization, chemosterilization, cytoplasmic incompatibility and hybrid sterility (Weidhaas, 1968). Commonly gamma irradiation from a cobalt-60 source has been used as the sterilizing agent and I also used this method in my studies.

However, a literature search failed to find more than a few references to use of the sterile insect release method in stored product pest control, either on laboratory or pilot scales. None included S. oryzae or R. dominica, the species I had selected for my studies.

The use of the sterile male release technique has been considered economically and ecologically disadvantageous for the control of the flour moth, Anagasta (Ephestia) kuehniella (Zeller) (Crook et al., 1960; Bull and Wond, 1963). Erdman (1974) felt that this technique was impractical

for the control of flour beetles in stored grain because of potential damage to the food by the released adults, the costs associated with shielding to stop reinfestation and the sanitary problems due to large numbers of added insects in the food. He also suggested that sterile females should be excluded from the sterile release method in storage pest control since the sterile females did not suppress population development, and since they destroy grain and cereal products. Pradhan et al. (1971) on the otherhand felt that this method was potentially most promising, provided it was properly planned and they suggested appropriate steps for its application.

In view of the paucity of informations in this area, and the obvious importance of the effect of radiation-induced sterility upon behaviour, I felt that any assessment of the value of the sterile male technique in stored products pest control required critical investigation of several fundamental factors. A programme designed to achieve adequate assessment of such a drastic technique would need to include all or many of the requirements listed by Knipling (1955, 1968). I therefore set out to determine the biology and innate capacity for increase in both S. oryzae and R. dominica, for although this had been studied by a number of workers, it seemed appropriate to repeat the work to ensure that I could manipulate the test animals satisfactorily and to find out whether they behaved similarly to those used in previous studies when reared within the system I wished to utilise.

I needed also to determine levels of radiation that produced sterility, the effect of radiation upon longevity and the ratio of sterile males to fertile males that resulted in cessation or depression of reproduction for the species. Finally, it was necessary to study certain aspects of the insects' behaviour. In particular it seemed desirable to understand the main factors involved in intra-specific communication, and

whether such processes were affected by radiation sterility. If intra-specific communication involved the production of pheromones it was conceivable that certain levels of radiation could affect either their production or the receptors involved in the behaviour-dynamics of insects. If indeed this was proven, then either mating or dispersal or both, may be seriously inhibited with great significance to the applicability of a sterile insect control system for such insects.

It seemed appropriate also to test insects within as natural conditions as possible. As most grain is stored in silos or columns of varying size, I chose to use columns of grain in which to study the dispersal of the sexes as a means of determining the presence of inter-sexual signals, the timing of their production and their effects.

The objectives were consistent with a proper assessment of the sterile insect release technique as a method for control of insect pests. The choice of the test animals was based upon (a) the intention of extending this method to the control of beetles inhabiting grain, (b) their availability, (c) their apparent ease of rearing, and (d) the fact that Birch (1945a, b, c, 1946, 1948, 1953) had done pioneering work upon them in this Institute.

The studies concerned with biology, innate capacity for increase, radiation sterilization and competitiveness between sterile and fertile males were completed upon both S. oryzae and R. dominica. The behavioural studies concerned with dispersal and intra-specific communication and the demonstration of sex pheromones were restricted to S. oryzae because of the ease of handling this species and the time available.

In view of the complex nature of these interrelated studies, I have organized the thesis into five sections as follows:

1. General Introduction
2. Biological Studies

3. Radiation Studies

4. Behavioural Studies

4.1.1 A radioisotope technique for studying the dispersal (movement) of S. oryzae in grain.

4.1.2 Studies on dispersal (movement) of S. oryzae adults in low density populations in grain.

4.1.3 Demonstration, collection, isolation and bioassay of pheromones of S. oryzae.

2. BIOLOGICAL STUDIES

2.1 Introduction

Both the rice weevil, Sitophilus oryzae (L.) and the lesser grain borer, Rhizopertha dominica (F.), the insects chosen for my research, have received considerable attention by biologists of many countries (Golebiowska, 1969; McFarlane, 1968; Singh et al., 1974). Nevertheless, some similar investigations appeared to be desirable in order to develop expertise in handling the species and to determine whether the test animals behaved in essentially the same ways as in previous studies utilising techniques I wished to employ (Birch, 1953). It therefore seemed appropriate to re-examine the biologies and the innate capacity for increase for both S. oryzae and R. dominica under conditions that would be used in most of my proposed experiments. This section reports results obtained relative to similar studies by other workers.

S. oryzae: The biology of S. oryzae and the effect of environmental conditions upon its population dynamics have been studied by a number of workers (Richards, 1944, 1947; Birch, 1945a, b, c, 1948, 1953; Howe, and Turner 1952). Hinds/(1911) studied the habits and life cycle of the insect while MacLagan and Dunn (1935) examined the effects of population density upon fecundity, utilization of food and frequency of copulation. Various influences upon development and fecundity have been reported (McFarlane, 1968; Golebiowska, 1969) including the effect of high yielding varieties of wheat (Singh et al., 1974).

Despite these and other researches, the rice weevil remains a major pest of stored products and continued efforts toward better understanding of factors influencing its behaviour and population dynamics are justified.

R. dominica: The biology of the lesser grain borer, R. dominica has been

investigated under a variety of conditions (Chittenden, 1911; Barnes and Grove, 1916; Schwardt, 1933; Potter, 1935) while the effects of crowding and competition with other species have been reported by Crombie (1942, 1944). The influence of environmental conditions on development, mortality of the immature stages, fecundity and longevity (Birch, 1945a, b, c, 1953; Howe, 1950; Golebiowska, 1969) have in certain cases resulted in defining the innate capacity for increase for this species under specified conditions (Birch, 1948, 1953).

2.2 Materials and Methods

2.2.1 General

All experiments in this section utilized a similar basic technique with minor differences for S. oryzae and R. dominica. A medium-soft wheat (Heron) was used throughout the experiments, and before use it was sterilized at 70°C for 3 hours to free it from insects and mites (Pixton, 1968). The temperatures at which experiments were conducted were 25 ± 0.5°C and 30 ± 0.5°C for S. oryzae and 30 ± 0.5°C and 33 ± 0.5°C for R. dominica. Relative humidity and moisture content of grain were maintained at 75% and 14.5% respectively. This was assisted by conducting experiments over saturated sodium chloride solutions in Fowler jars covered with air-tight glass lids (Rockland, 1960) and by selecting wheat from bulk supplies equilibrated to a standard moisture content, which was tested from time to time. There is a relationship between relative humidity of air and grain moisture content (Gay, 1946). When grain, arranged in a few layers in plastic vials ventilated at both ends, is exposed to air with a 75 percent relative humidity at 30°C, the moisture content of the grain will approximate 14.5 percent. At all other experimental temperatures, the m.c. of grain tested was essentially the same as it was at 30°C.

When the moisture content of the grain had to be raised to the required moisture level, its actual moisture content was determined and water was added according to the following formula:

$$\frac{100 - \% \text{ of present moisture}}{100 - \% \text{ of desired moisture}} - 1 = F$$

Then: F x gm of grain = ml of water required to bring the grain to the required moisture content. The "tempered" grain was then stored in air-tight containers and maintained at 40°C (Harein and Soderstrom, 1966) for one week. The grain was then subjected to the experimental temperature for stabilization and was then tested by oven drying samples before use of the remainder. The test method involved selecting 5 random aliquots, each of 20 gms and drying the sample at 120°C for 4 hours. It was assumed that only water was lost and therefore the moisture content was calculated as percentage of the weight before oven drying (Pixton, 1967).

In all experiments, unless otherwise stated, a "standard rearing chamber" was used. It was constructed from a styrene tube 62 mm long and 25 mm in diameter. The open ends were fitted with wire gauze (bottom) and with ventilated lids (top).

Adults of S. oryzae were sexed on the characters of the rostrum (Reddy, 1951; Halstead, 1963). The sexes of R. dominica were separated either during the pupal stage on the genital papillae (Potter, 1935) or in the adult stage on colour differences of the abdomen (Stemley and Wilbur, 1966).

A binocular microscope (35 x magnification) was used for all observations. Individuals were classed as dead if they failed to move when gently and repeatedly touched with a camel hair brush or, if quiescent, when they became discoloured or shrivelled.

2.2.2 Insect stock cultures

Cultures of both test insects were obtained from the South Australian Department of Agriculture and were maintained at 30 ± 0.5°C and 75 percent relative humidity in wheat to provide bulk cultures for experiments.

2.2.3 Experimental cultures

S. oryzae: These cultures were prepared by adding 200 adults, aged 1-2 weeks, to 200 gm of wheat grain in 250 gm plastic cups, the bottoms of which were replaced with wire gauze and the tops covered with ventilated lids. Cultures were maintained in the standard environmental conditions explained above. After one week of oviposition, the adults were removed and discarded. Under these conditions, adults began emerging about 4-6 weeks after the cultures were established, the earlier emergences being associated with the higher temperature at which the insects were reared. Peak emergence was usually one week after the initial emergence at both temperatures. The contents of the culture cups were sieved to separate insects from wheat. For critical tests, involving adults of specified ages, the insects were collected every 4 hours over the period from 9 a.m. to 9 p.m. each day.

R. dominica: Cultures of *R. dominica* were prepared in the same way as those of *S. oryzae* except that the medium was a 4:1 mixture of whole wheat and partially crushed grain (Matin and Hooper, 1974). The crushed grain was added to provide better conditions for oviposition (Birch, 1945c). New generations started emerging 4-5 weeks from establishment, the earlier emergences being in the 33°C cultures. As in *S. oryzae*, the peak emergence was about one week after initial emergence at both the temperatures. Insects were separated from the media by a double sieving. The first removed the crushed medium and the second separated the beetles

from their frass. Difficulty was experienced in determining the exact age of collected adults because they re-entered grain following emergence and were not readily extracted by sieving. Therefore, when adults of known age were required, parent stock of adults were placed in a medium of ground wheat mixed with whole wheat kernels (1:9 ratio) as described by (Strong and Sbur, 1964; Strong et al., 1967). To ensure collection of vigorous insects, I placed adults in the centre of a large petri dish which was covered to prevent their escape. Only those insects that moved quickly to the edge of the dish were used for experiments (Strong et al., 1967).

2.2.4 Studies on the developmental period and the mortality of immature stages

S. oryzae: In order to determine the developmental period and the mortality of the immature stages, I segregated 10 replicates each of 20 eggs, 0-24 hours old. They were obtained by allowing females, 1-2 weeks old, to oviposit on grain in 25 mm x 12 mm styrene tubes with open ends covered as previously described (see Materials and Methods). The cultures were reared under the standard conditions described earlier for 24 hours. Then the insects were removed and the wheat was checked for eggs. This was repeated until enough eggs for experiments were obtained. As a rule one weevil completes development in one grain, the rest being eliminated by cannibalism during the immature stages (Howe, 1952). This competition within a grain slows the development of the survivor (Birch, 1945a). So only one egg was allowed to remain in each grain and any others were destroyed with a needle. The 10 replicates of 20 eggs were maintained under the standard rearing conditions until emergence of the adults began i.e. after about 5 weeks at 25°C and 3 weeks at 30°C respectively. Records were made daily until emergences were completed and the mean developmental periods calculated. Total mortalities of the immature stages were

calculated as a percentage of the number of eggs present at initiation of each experiment. The formula for calculation was:

$$\frac{F - E}{F} \times \frac{100}{1}$$

where F is the initial number of eggs and E the number of adults that emerged.

R. dominica: The method used here was the same as for S. oryzae except for food, temperatures and collection of eggs. A 4:1 mixture of whole wheat and crushed grain was used as the culture medium. The temperatures were 30°C and 33°C and eggs were obtained by placing females, 1-2 weeks old, on the medium in 50 mm diameter styrene petri dishes with ventilated lids. The bottoms of the petri dishes were replaced with wire gauze.

Adults were removed from the petri dishes after 24 hours of oviposition and added to other batches of media. This continued until enough eggs had been obtained, then adults were discarded. The eggs along with media were incubated at the experimental temperatures for 3 days to induce development of the egg cuticle so that damage during handling was reduced (Birch, 1945a). They were then separated with a needle and transferred to standard rearing containers of scalpel cut grains. As far as possible eggs were placed into the scalpel cut on each grain thus reducing the chances of mortality following eclosion (Birch, 1945b). Each rearing container had 20 eggs kept under standard rearing conditions. The duration of development and the mortality during the immature stages were calculated in the same way as for the rice weevil.

2.2.5 Age-specific fecundity

S. oryzae: Age-specific fecundity studies utilized newly emerged adults. Three replicates of 2 pairs of insects were established, each replicate having 40 grains of wheat or a density of one insect per 10 grains, which largely overcame crowding effects (MacLagan and Dunn, 1935). Insects

and wheat were kept in standard rearing containers under the standard rearing conditions. Wheat was changed every week and counts of eggs present were also made weekly by microscopically locating the gelatinous egg plugs on the seed coat. In addition, mortality of the adults was recorded.

R. dominica: Three replicates of 5 pairs of newly emerged adults were segregated at each temperature and provided with 6 grains of scalpel cut wheat per insect. This density overcame the effects of crowding (Crombie, 1942). The insects and wheat were maintained under standard rearing conditions with grain being changed and eggs counted weekly; eggs were found mainly in the scalpel cut or under the loose testa of each grain.

2.2.6 Longevity of adults

S. oryzae: A total of 200 newly emerged adults, 0-24 hours old, were used at each experimental temperature. Each lot of 200 adults was divided into 10 replicates of 10 pairs of insects, each set being provided with 7 gm of wheat, approximately equivalent to a density of one insect per 10 grains. The insects were reared in the same manner as for previous experiments. Each set was examined once a week for dead insects, which were removed, sexed and counted. Throughout the experiment the population density in each container was kept constant, as far as possible, by replacing dead insects with living ones from the highest numbered tubes, to bring the others back to a total of 20 insects per tube. Thus the number of rearing sets became successively reduced during the experiments but the density of insects remained reasonably constant in residual sets (Birch, 1953). Grain was completely replaced weekly.

R. dominica: The procedure for this species was the same as for S. oryzae, the density was maintained at one insect per 6 grain-equivalents of wheat medium being a 4:1 mixture of whole and damaged grain.

2.3 Results and Discussion

2.3.1 Developmental period and mortality of immature stages

S. oryzae: The time required to develop from egg to adult was about 40 and 28 days at 25°C and 30°C respectively (Table 2.1) and the mortality of the immature stages under the same conditions was 18.5% and 13% respectively (Table 2.2). The developmental period at 30°C in the present study was close to those of other workers (Birch, 1945a; Howe, 1952; McFarlane, 1968; Golebiowska, 1969) but it was longer by 5-12 days at 25°C than in most other reported studies (Birch, 1945a; McFarlane, 1968; MacLagan and Dunn, 1935; Singh *et al.*, 1974). It was about

Table 2.1 Developmental periods for *S. oryzae* and *R. dominica* under controlled conditions.

Species	Temp. °C	% Relative humidity	% Moisture content of grain	Mean Developmental period (Days ± S.E.)
S. oryzae	25	75	14.5	39.94 ± 0.15
	30	75	14.5	27.96 ± 0.14
R. dominica	30	75	14.5	34.27 ± 0.26
	33	75	14.5	26.58 ± 0.15

5 days less than the period determined by Richards (1944). The mortality figure at 30°C was close to that reported by Birch (1945b) but again at 25°C my results considerably differ from his possibly due to differences in variety of wheat used as Singh *et al.* (1974) found in their studies.

Table 2.2 Mortalities of immature stages of S. oryzae and R. dominica under controlled conditions.

Species	Temp. °C	% Relative humidity	% Moisture content of grain	Number		% Mortality
				eggs	adults	
S. oryzae	25	75	14.5	200	163	18.50
	30	75	14.5	200	174	13.00
R. dominica	30	75	14.5	200	140	30.00
	33	75	14.5	200	148	26.00

R. dominica: In R. dominica, the duration of development under the conditions used was about 34 and 27 days at 30°C and 33°C respectively (Table 2.1) and 30% and 26% were the mortalities during development at the same temperatures (Table 2.2). These results approximate those reported by Birch (1945a), Potter (1935), Howe (1950), and Crombie (1944) but are at variance with those of Golebiowska (1969) who reported a very long developmental period (67.9 days at 28°C).

Mortality of the immature stages at both the temperatures was higher in the present study than was reported by Birch (1945b) and Howe (1950).

2.3.2 Age-specific fecundity

S. oryzae: The weekly rate of oviposition during the life of the insect at each temperature is shown in (Appendix 2.1). The rate of oviposition reached its peak in the 3rd week at 30°C while at 25°C, higher oviposition rates extended from the 2nd to the 5th week. Thereafter oviposition

gradually decreased until completion by the 14th week. The mean number of eggs laid per female during life at 25°C and 30°C were 187 and 268 respectively (Table 2.3).

Table 2.3 Fecundities of S. oryzae and R. dominica under controlled conditions. (Data presented as average number of eggs produced per female during its life).

Species	Temp. °C	% Relative humidity	% Moisture content of grain	Eggs per female
S. oryzae	25	75	14.5	187.36
	30	75	14.5	267.59
R. dominica	30	75	14.5	671.05
	33	75	14.5	402.60

These results provide lower measures of fecundity and oviposition than were recorded by Birch (1945c, 1953) under slightly different conditions.

R. dominica: The mean fecundity of R. dominica was 671 and 403 at 30°C and 33°C respectively (Table 2.3) which reversed the trends obtained by Birch (1953) when the maximum eggs were laid by insects kept at 32.3°C. The production of eggs with time by R. dominica (Birch, 1945c) tends to show no distinct peak at temperatures below 34°C. This observation was also supported by Howe (1950). Oviposition, which started in the first week following establishment of my cultures, continued for 21 weeks at 30°C and for 17 weeks at 33°C. There was a higher ovipositional rate in the first 13 weeks at 30°C and the first 8 weeks at 33°C than later in the respective periods (Appendix 2.2).

2.3.3 Longevity of adults

S. oryzae: The mean longevities of the sexes as Birch (1953) had found earlier, are similar. My results (Appendices 2.3 and 2.4) show a tendency for females to die faster than males in early life in the 30°C cultures. In the first 5 weeks of culture 14% of the females died compared with 2% of males. However about 50% mortality was recorded in the eleventh week for females and twelveth week for males. Less variation in mortality of the sexes occurred at 25°C where the mean longevities (weeks) were 15.3 (females) and 13.6 (males) respectively (Table 2.4) and 50% mortality was recorded at similar times as those in the 30°C cultures. These longevities compare with 17.05 weeks (females) and 16.58 weeks (males) at 29.1°C (Birch 1953), 9 weeks at 25.5°C for both sexes (McFarlane 1968) and 14 weeks for both sexes reared on rice by Prevett (1960) at a mean temperature of 27°C.

Table 2.4 Mean length of adult life for S. oryzae and R. dominica.

Species	Temp. °C	% Relative humidity	% Moisture content of grain	Mean longevity (Weeks ± S.E.)	
				Male	Female
S. oryzae	25	75	14.5	15.3±0.75	13.6±0.64
	30	75	14.5	12.9±0.58	11.2±0.55
R. dominica	30	75	14.5	18.7±0.76	17.2±0.79
	33	75	14.5	12.4±0.58	12.1±0.60

R. dominica: There were similar longevities and survival rates for both males and females at each experimental temperature (Table 2.4 and Appendices 2.5 and 2.6). At 33°C the mortality throughout the experiment was approximately linear at between 5% and 10% per week with generally lower

values in the first 4 weeks and 50% dead by week 12. Mortality at 30°C was initially lower than at 33°C, > 70% surviving for 12 weeks with male survival superior. Longevity at 30°C was greater than at 33°C (Table 2.4). The longevity figures for R. dominica males and females reported by Birch (1953) at 32.3°C were 19.67 and 17.2 weeks respectively which are higher than recorded herein (Table 2.4). Howe (1950) also reported lower longevities for R. dominica than those obtained by Birch (ibid.).

2.4 Innate capacity for increase

Birch (1948) defines the intrinsic rate of natural increase (i.e. innate capacity for increase) as "the rate of increase per head under specified physical conditions, in an unlimited environment where the effects of increasing density do not need to be considered".

The innate capacity for increase is a useful statistic that summarizes the aspects of reproduction: the fecundity rates, the age of the female, the speed of development and longevity of the female. The innate capacity for increase is to some extent characteristic of the species of animal, is affected by its environment and therefore must be defined in relation to a particular set of environmental conditions (Andrewartha and Birch, 1954).

Since it was shown that the fecundity rates of both S. oryzae and R. dominica are affected by temperature, it is to be expected that their innate capacities for increase would also change as was shown by Birch (1953).

In order to calculate the innate capacity for increase for any species, it is necessary to know (a) life table, (b) duration of development of the immature stages, and (c) age-specific fecundity rates (Birch, 1953).

The data for developmental period, age-specific fecundity and the longevity of S. oryzae and R. dominica were worked out earlier in this

section and are summarized after Birch (ibid.) and Andrewartha and Birch (1954) in Fig. 2.1 (S. oryzae) and Fig. 2.2 (R. dominica).

From these data, the capacity for increase, r_c's (Laughlin, 1965) for each temperature and for each insect were calculated. Because of the overlapping generations, however, r_c was an underestimate of the innate capacity for increase. Consequently r_m was calculated using the method described by Birch (1948). The values for r_m and r_c for both S. oryzae and R. dominica at the experimental temperatures are shown in Table 2.5.

Table 2.5 Innate capacities for increase for S. oryzae and R. dominica.

Species	Temp. °C	% Relative humidity	% Moisture content of grain	r_c	r_m
S. oryzae	25	75	14.5	0.400	0.468
	30	75	14.5	0.535	0.666
R. dominica	30	75	14.5	0.419	0.607
	33	75	14.5	0.535	0.738

The r_m values for S. oryzae at 25°C and 30°C were 0.468 and 0.666 respectively while for R. dominica at 30°C and 33°C, the respective values of r_m were 0.607 and 0.738. For both species the value of r_m increases with temperature under the conditions studied here. Birch (1953) reported higher values for innate capacities of increase under various environmental conditions for both S. oryzae and R. dominica than found in the present study but none of his conditions were identical with those I used. Thus

Figure 2.1 Life tables (lx) and age specific fecundities (mx) for S. oryzae at 25°C and 30°C.

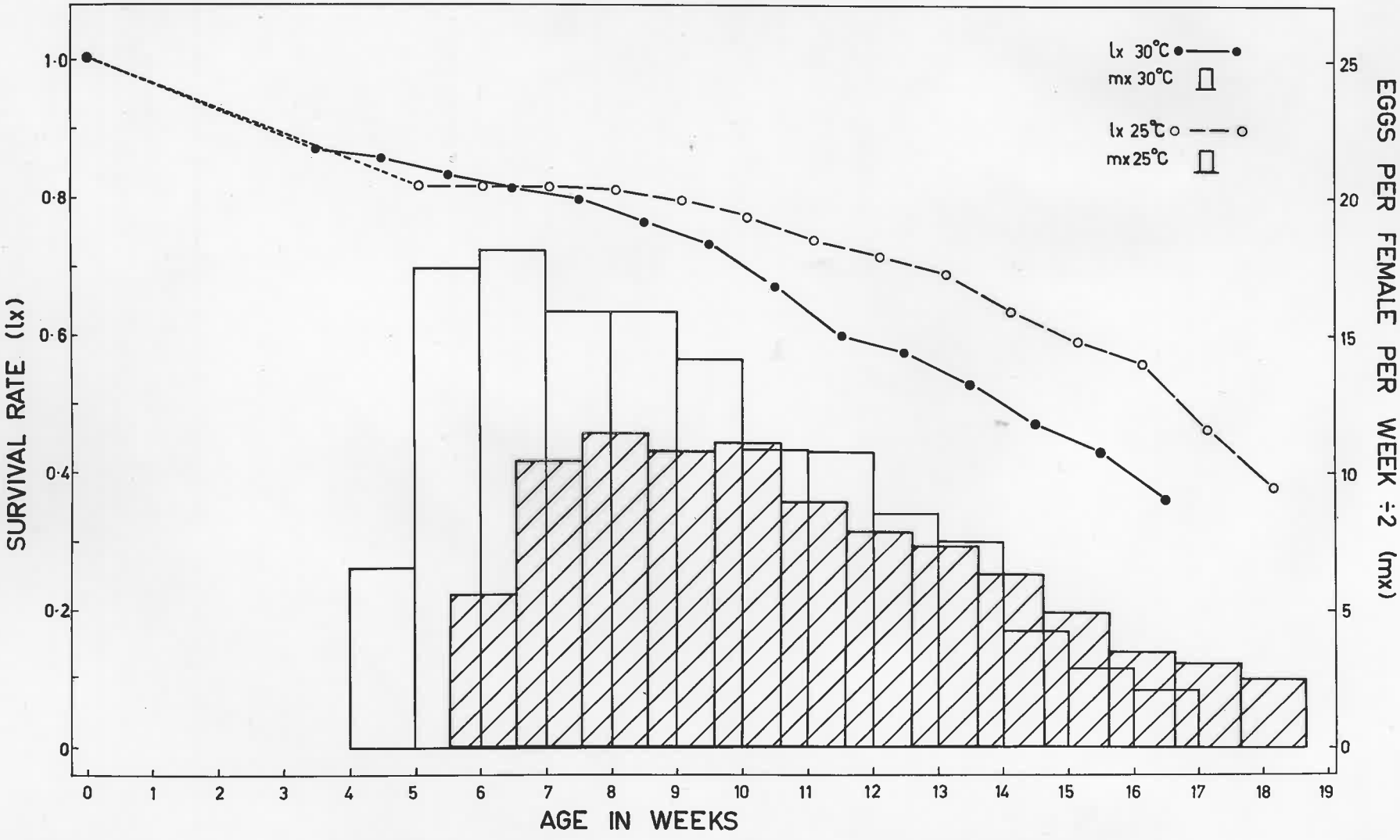
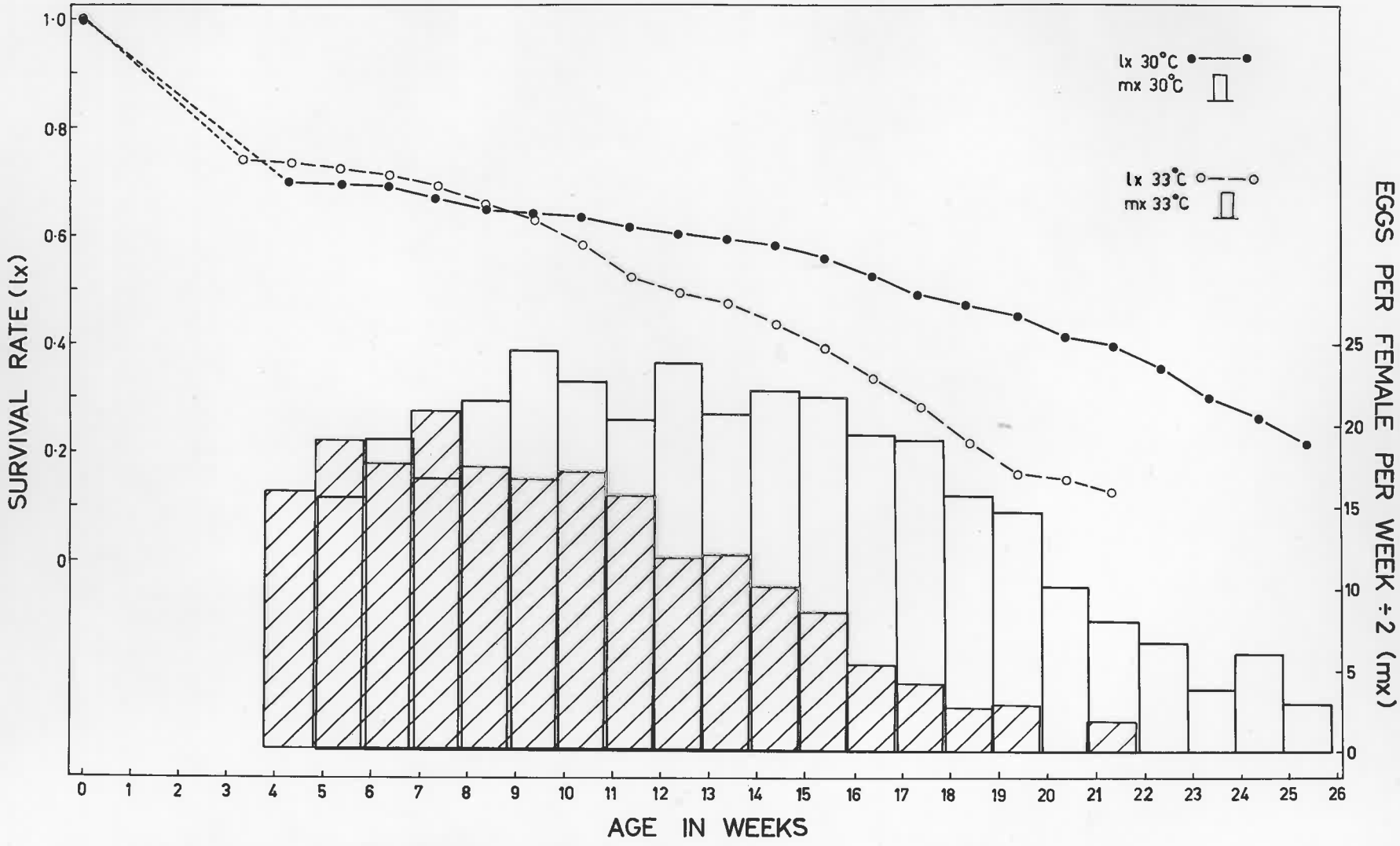

lx 30°C
mx 30°C
lx 25°C
mx 25°C
SURVIVAL RATE (lx)
EGGS PER FEMALE PER WEEK ÷2 (mx)
AGE IN WEEKS
1·0
0·8
0·6
0·4
0·2
0
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Figure 2.2 The life tables (lx) and age specific fecundities (mx) for R. dominica at 30°C and 33°C.


lx 30°C
mx 30°C
lx 33°C
mx 33°C
SURVIVAL RATE (lx)
EGGS PER FEMALE PER WEEK ÷ 2 (mx)
AGE IN WEEKS
1·0
0·8
0·6
0·4
0·2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
25
20
15
10
5
0

the lower values for r_m obtained by me can be explained by the longer developmental periods in some cases and to slight differences in rearing conditions and lower fecundity in other cases. Evans (1977) under similar environmental conditions and temperature to those used by Birch (1953) recorded higher values for r_c in S. oryzae.

2.5 Conclusion

These studies though not directly comparable with those of other workers, gave results that fell within the ranges reported and referred to above. They demonstrated that the two species were behaving similarly under specified rearing conditions to those reared in various parts of the world by other entomologists already mentioned above. The data obtained provided a sound basis for subsequent studies.

3. RADIATION STUDIES

3.1 Introduction

Irradiation of insects has received wide attention including fundamental studies on the effects of radiation upon chromosomes, experimental approaches aimed at developing techniques for field use of this biological effect and successful application of it to insect-pest eradication and control. These controls have been effective in both limited or localized and country-wide programmes.

The most promising application of radiation in economic entomology so far has been the "sterile insect release" technique, the principles of which were first postulated by Knipling (1955). It gained world-wide interest through its successful application to the screw worm fly, Callitroga (Cochliomyia) hominivorax (Cqrl.) (Baumhover et al., 1955; Lindquist, 1955). Since then the method has been applied to different pests in many parts of the world, either in small or medium-sized field tests (LaChance, 1974) but these have not included stored products pests.

There is now an extensive literature on the sterile insect release method as it applies to controls or attempted controls for a wide variety of insects.

The first attempt to examine this method on a stored products pest was that of Crook et al. (1960) on Anagasta (Ephestia) keuhniella (Zeller). Since then a few further laboratory trials have been made on some stored products pests. These included Tribolium castaneum (Herbst) (Pradhan et al., 1971; Erdman, 1974), Tribolium confusum (Duval) (Erdman, ibid.); Plodia interpunctella (Hubner) (Ahmed et al., 1976 a, b), and a theoretical evaluation of sterile male technique with fully or partially

sterile males in Cadra (Ephestia) cautella (Walker) (Brower and Tilton, 1975). When I began these studies, no such experiments had been reported for either S. oryzae or R. dominica and it seemed appropriate to assess the potentialities of the sterile insect release method on these two important pests of grain, at least at the laboratory level.

In such an assessment, in addition to other basic requirements (Knipling, 1955, 1964, 1968), one has to determine the radiation sterilizing dose for each species, and its effect upon longevity and sexual competitiveness of sterile male insects compared with fertile males of the same age.

Despite the lack of attempts to control S. oryzae and R. dominica by the sterile insect release technique, a number of studies on irradiation of different stages of the life cycles of these beetles have been completed (Hassett and Jenkins, 1952; Proctor et al., 1954; Cornwell et al., 1957; Cornwell and Morris, 1959; Dennis, 1961; Pendlebury et al., 1966; Huque, 1963; Tilton et al., 1966; Watters and MacQueen, 1967; Matin and Hooper, 1974).

Other associated studies have included the loss of weight by wheat infested with gamma-irradiated S. oryzae and R. dominica (Brower and Tilton, 1973), the effect of modifying temperature on the survival of R. dominica following irradiation (Singh and Liles, 1972), and the examination of radiation-resistance in S. oryzae (Brower, 1974; Matin, 1975).

No assessment of the effect of irradiation upon sexual competitiveness between sterile and fertile males on either species has so far been reported.

Despite the above reported studies, it is essential to re-determine the radiation sterilization doses and their effects on the insects upon which new studies are to be made. This is emphasised by differing reports of these earlier workers and also because the radiation effects may

vary with different strains of a species (Holt, 1975), with variation in geographical location of the species (Soliman, 1973) or even with changes in experimental methods, such as variation in temperature (Singh and Liles, 1972; Soliman, 1973).

Experiments were designed to determine the radiation doses needed to sterilize each species and to assess the effects of such doses upon longevity and competitiveness of adults.

In addition, studies on the effect of varying the densities of males to females upon actual fecundity or progeny produced were completed for non-irradiated insects as well as for cultures that included differing proportions of sterilized males to fertile males; the objective was to obtain a comparative measure of the effect of male-dominated sex ratios *per se*, and the combination of this and male sterility. There is little doubt that, in the sterile insect release method of population control, part of the reduction in population growth is due purely to density effects.

3.2 Materials and Methods

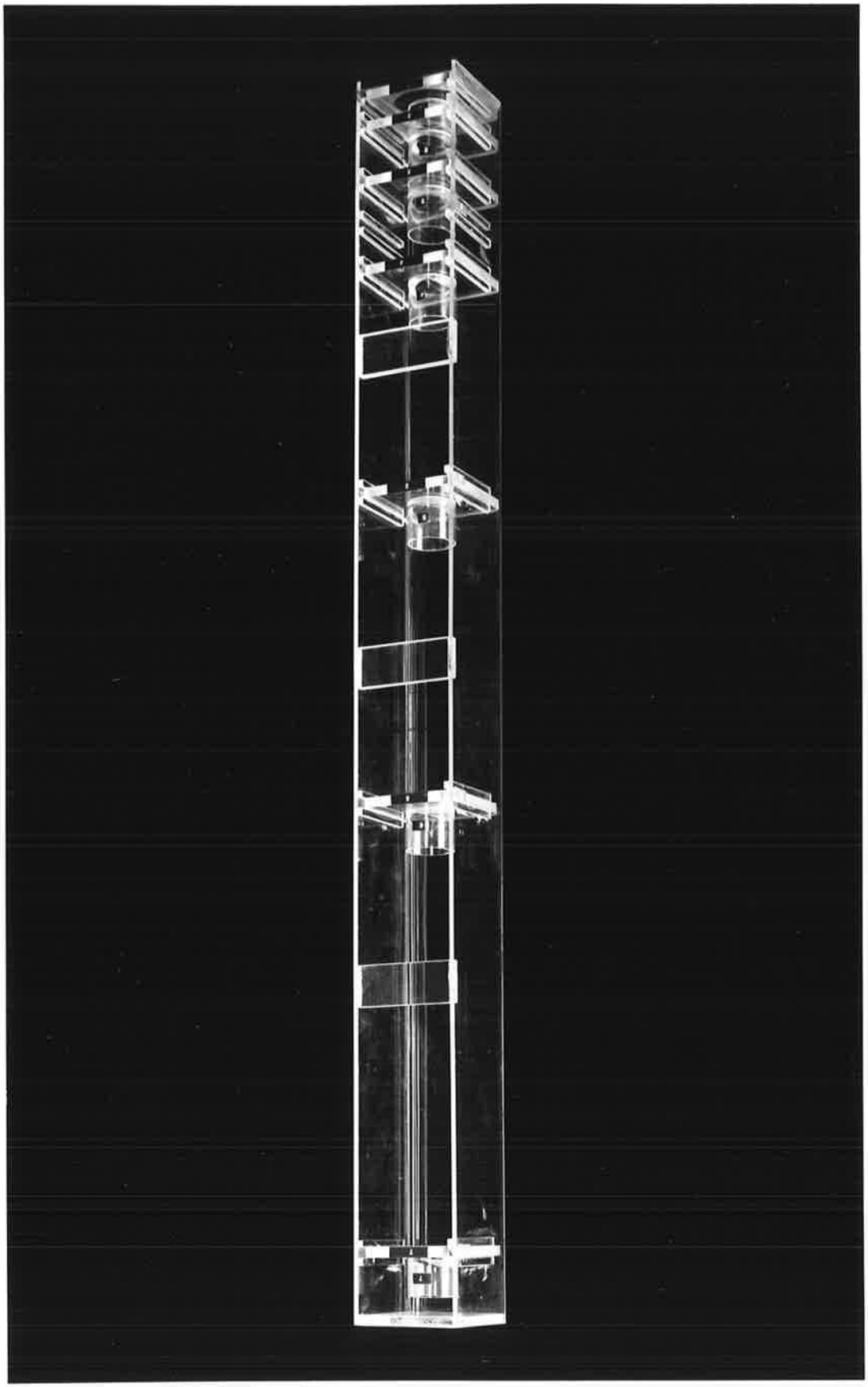
3.2.1 General

All experiments were conducted at 30 $\pm$ 0.5^{o}C, 75% relative humidity and with wheat (Heron) of 14.5% moisture content. The standard rearing container described in Section 2 was used for each experiment.

3.2.2 Irradiation technique

Irradiations of adults were carried out in the 'Orbitron', a 1500 ci ^{60}Co unit, in the Radiotherapy Department at the Royal Adelaide Hospital. A perspex frame (Fig. 3.1) 75 cm long with a 6.5 cm square cross section was used for irradiation of all specimens (Robertson, 1973). It was clamped into position vertically above the source. The frame with

Figure 3.1 A perspex frame for holding specimen vials at various distances from the ^{60}CO source (after Robertson, 1973).



a number of shelves spaced at various distances from the base was constructed to provide a range of dose-rates that could be used at different time intervals to get approximately similar dose rates each time.

The shelf dose-rate was calibrated by Thermoluminescence dosimetry (Worthley, 1974). The range of dose-rates obtained were from 1916 rads*/minute nearest the source (shelf 1) to 704 on shelf 4. These values were adjusted over the period of my experiments according to the decay rate of 1.2%/month for the ^{60}Co source. It was estimated that the accuracy of calibration for the dose rates was of the order of $\pm$ 3%.

The insect specimens were placed in 25 mm (1 inch) polystyrene vials and, for relatively uniform irradiation, were kept within 5 mm of the bottom of the vials with ventillated plugs during exposure to the source.

Dose-rates used varied between 704 and 987 rads/minute and similar dose rates were maintained for specific experiments. The doses used were all single and acute.

3.2.3 Determination of sterilizing doses

The sterilizing doses for both S. oryzae and R. dominica had been determined previously by other workers (Cornwell and Morris, 1959; Cornwell et al., 1957; Hassett and Jenkins, 1952; Pesson, 1963; Tilton et al., 1966; Pendlebury et al., 1966; Matin and Hooper, 1974). Nevertheless preliminary experiments were conducted to re-examine and re-check the sterilizing doses for both insects.

S. oryzae: Cornwell and Morris (1959) reported that adults of this species were less susceptible to the effects of radiation at certain times. For this reason 5-8 day-old beetles were used in my experiments to determine the sterilizing dose. The sexes were irradiated individually after being

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* rads = 100 ergs/gm

assigned to 10 replicates of one pair for each treatment. Seven treatments: 4, 6, 8, 9, 10, 11 and 12 Krads provided a range within which the sterilizing dose was expected to lie.

Immediately after irradiation single pairings of males and females were made under the standard conditions, each pair having 4 gm of wheat. Six weeks after establishment of the cultures, they were checked for progeny. The sterilizing dose was determined as the lowest dose at which no offspring were produced.

R. dominica: The methods used for R. dominica were similar to those for S. oryzae except that the rearing medium was a 4:1 mixture of whole wheat and crushed grain and the dose range was 6, 8, 10, 11, 12, 13 and 14 Krads. Cultures were checked for progeny 7 weeks after their establishment. The sterilizing dose was determined in the same way as for S. oryzae.

3.2.4 Survivals of the sterilized adults

Because the effects of radiation are influenced by the age of the insects (Wharton and Wharton, 1959; Cornwell and Morris, 1959; Baxter and Tuttle, 1957; O'Brien and Wolfe, 1964), survivals of the sterilized adults of both test insects, were determined for two age groups (1-7 days, and 8-14 days, old) over an experimental period of 30 days.

S. oryzae: Groups of 100 pairs were irradiated at the sterilizing dose when they were either 1-7 days or 8-14 days old. The separate age-groups were tnen assigned to treatments each having 10 replicates of 10 pairs of beetles, each replicate being provided with 7 gm of wheat or about one insect to 10 grains of wheat. Kept under the standard rearing conditions, the replicates were examined for mortality every 2 days. The dead were removed, sexed and counted and food was replaced weekly.

Controls consisted of the same number of replicates of non-

irradiated beetles of the same age groupings.

R. dominica: The differences between the design of experiments for this species and for S. oryzae were:

Food: 4 to 1 mixture of whole and crushed grain - quantity equivalent to about 6 grains of wheat per insect.

3.2.5 Competitiveness between sterile and fertile males

Experiments were designed to study the effects of certain ratios of sterile to fertile males on the population increase of both S. oryzae and R. dominica. The general plan of the experiments is shown in Table 3.1 for both species.

Table 3.1 General plan for competitiveness experiments for both S. oryzae and R. dominica.

Treatments	Number of sterile males	Number of fertile females	Number of fertile females
0*	-	1	1
1	1	1	1
2	2	1	1
3	4	1	1
4	8	1	1
5	16	1	1
6	32	1	1

* Control

S. oryzae: Beetles between 5 and 8 days-old were sterilized and individuals were selected and assigned to the various treatments shown in Table 3.1. Each treatment was replicated 10 times and each replicate, provided with 12 gm of wheat, was kept under standard rearing conditions for 6 weeks. The insects were then removed and counted and increase in population is determined by subtracting the original number of beetles per replicate.

R. dominica: The only differences between the methods used for this species and for S. oryzae were:

Food: a 4 to 1 mixture of whole wheat - amount 10 gm per replicate.

Assessment: Assessment of population growth was made 7 weeks after establishment of the treatments and controls.

3.2.6 Effect of male density on the growth of insect populations

Population growth in insects is affected by the sex ratio (Crombie, 1942) and therefore I felt it essential to determine the effect of the same range of male to female ratios as was used in the different treatments in the sterile male competitiveness experiments. Such data would provide an estimate of the effect of this factor on the reduction of natural population growth. The general plan and procedure of this experiment was similar to those shown in Table 3.1, except that here all males were fertile.

Observations on the population increase in both S. oryzae and R. dominica were made 6 and 7 weeks respectively after the initial establishment of the cultures. The calculation to determine the number of progeny produced in each treatment was made in a similar way to that used in the "Competitiveness experiments".

3.3 Results and Discussion

3.3.1 Determination of sterilizing doses

S. oryzae: No progeny was produced at doses of 10 Krads or more. This result is in agreement with Pesson (1963) and very close to that of Cornwell and Morris (1959) who reported a slight residual fertility (0.008 of control) at 11,360 rep.* However, Cornwell et al. (1957) reported 6,000 rep as the sterilizing dose for S. oryzae while Hassett and Jenkins (1952) considered that a dose of 16,000 r** was required to produce complete sterility in adults of nine species including S. oryzae.

R. dominica: The adults of R. dominica irradiated at 11 Krads produced only one offspring/10 replicates, but at 12 Krads and higher no progeny were produced. Matin and Hooper (1974) obtained similar results. They determined 11.5 Krads as the sterilizing dose for R. dominica. Pendlebury et al. (1966) reported some reproduction with a dose of 11.2 Krads but none with 13.6 Krads or higher and they calculated 99.9 percent sterility at 11.24 Krads. Tilton et al. (1966) found only 4 adult progeny from 30 adults treated with 13.2 Krads but none from those given 17.5 Krads or more.

3.3.2 Survivals of the sterilized adults

The survival experiments for both S. oryzae and R. dominica were originally designed to continue for 30 days but sterile adults of S. oryzae died earlier than this. Therefore experiments with S. oryzae were continued until the last insect died while for R. dominica they continued for the full period.

S. oryzae: Sterile adults of both the age groups (1-7 days and 8-14 days

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* rep = 93 ergs/gm of tissue

** r = roentgen = 84 ergs/gm of air or, 93 ergs/gm tissue = rep.

old) and sexes died within 20 days after irradiation (Fig. 3.2 and 3.3) compared with almost complete (98% and 100%) survival in the 1-7 day-old male and female controls and 89% and 93% in the 8-14 day-old controls respectively, during the same period. Few sterile insects died within the first week of the experiments but over 50% had died by day 12 in both age groups. The mean longevities of sterilized insects are given in Table 3.2.

Table 3.2 Mean longevities of S. oryzae sterile males and females of two age groups (1-7 days and 8-14 days).

Mean longevity (Days ± S.E.) 1-7 day-old group		Mean longevity (Days ± S.E.) 8-14 day-old group	
Males	Females	Males	Females
11.46 ± 0.26	11.30 ± 0.27	11.60 ± 0.20	10.00 ± 0.18

Tests of significance were applied to these data and indicated that the survivals of the males of the two age groups were not significantly different at the 5% level of probability. The same result was obtained for the females of the two age groups.

The data on survival of S. oryzae showed similarities to the results of Cornwell and Morris (1959) who reported that all adults died within 3 weeks of treatment with 11,360 rep. Pesson (1963) also estimated that 80% - 90% died within 18 days of irradiation with a dose of 10 Krads or more. Cornwell et al. (1957) recorded 100% mortality of the adults in 20 days even at a dose of 6,000 rep while Hassett and Jenkins (1952) showed a total mortality of the adults within 12 days of being given 16,000 r.

Figure 3.2 Comparative survivals of sterile and fertile males and females of S. oryzae (beetles aged 1-7 days when irradiated).

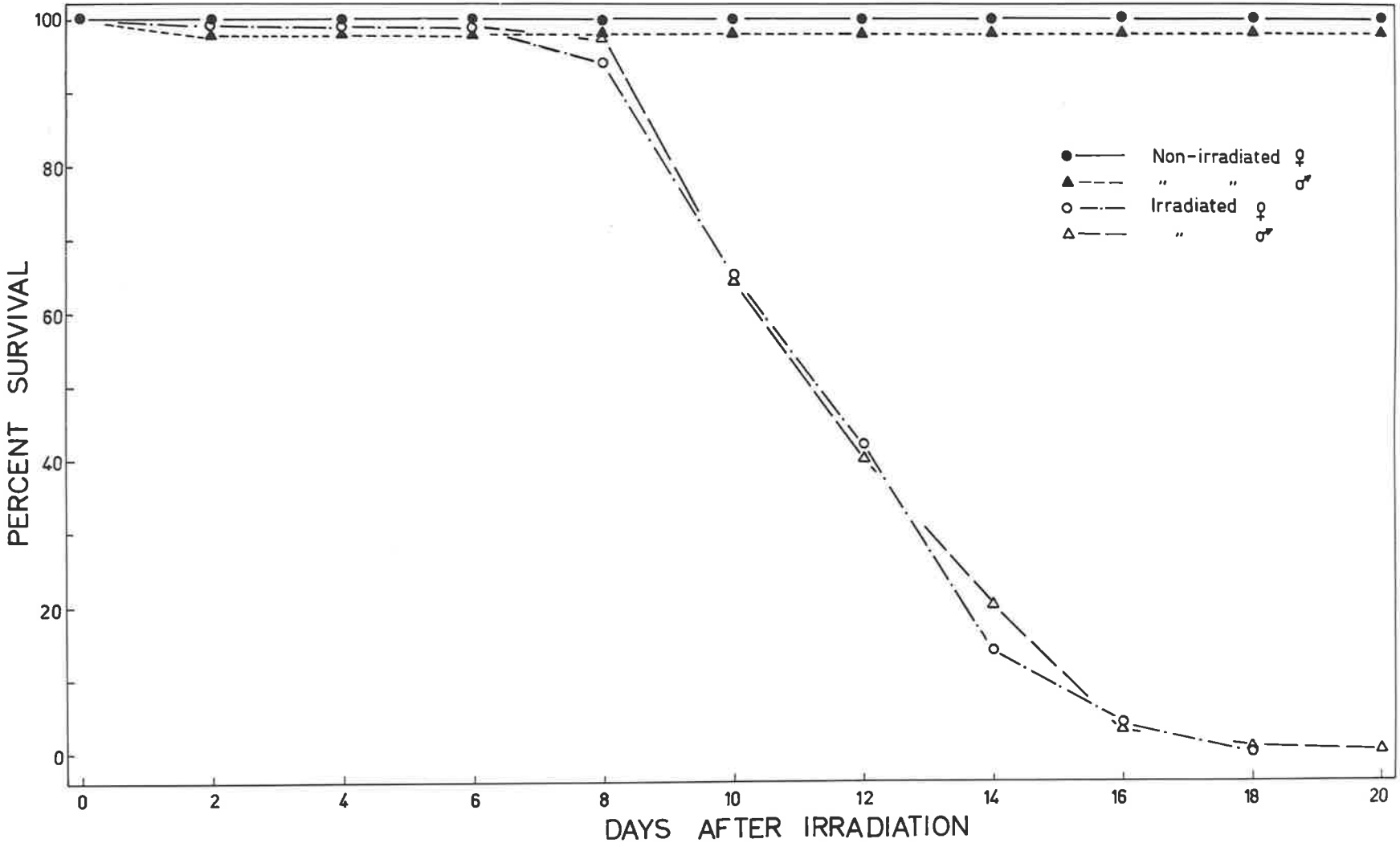
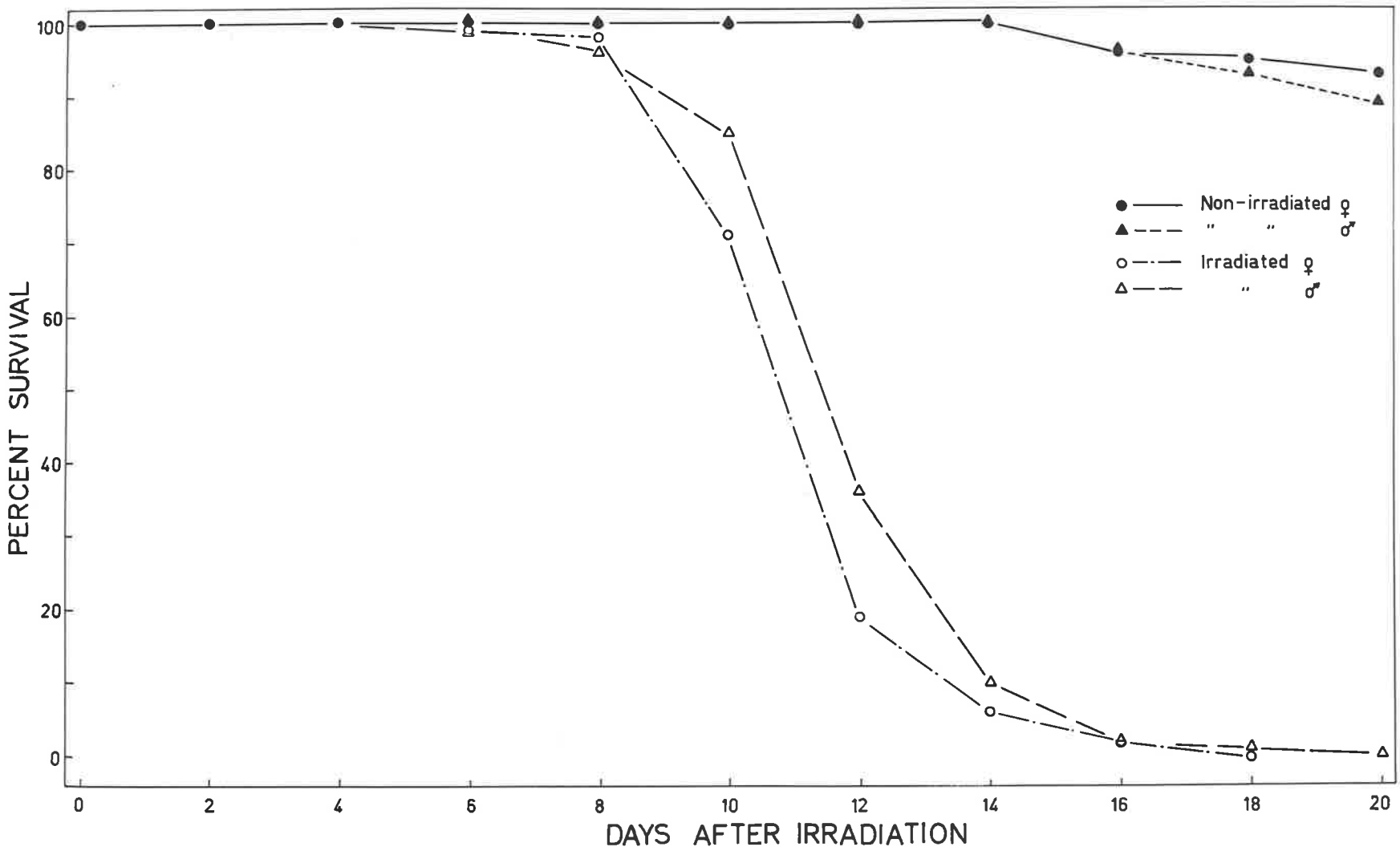

PERCENT SURVIVAL
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
DAYS AFTER IRRADIATION
Non-irradiated ♀
" "
Irradiated ♀
" ♂

Figure 3.3 Comparative survivals of sterile and fertile males and females of S. oryzae (beetles aged 8-14 days when irradiated).


PERCENT SURVIVAL
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
DAYS AFTER IRRADIATION
Non-irradiated ♀
" ♂
Irradiated ♀
" ♂

R. dominica: Adults of R. dominica were more resistant to irradiation than S. oryzae. Total mortality did not occur in any of the replicates and in either of the age groups even 30 days after irradiation at 12 Krads (Fig. 3.4 and 3.5). Female survival was higher in both age groups. About 50% of males died within 14 days in 1-7 days-old insects and within 18 days in the older age group. Mortalities in controls were 5% and 6% for males and females respectively in the younger and 8% and 16% respectively in the older of the age groups tested. There was again no significant difference in survival between the males or between the females of the two age groups.

The results on survival of the sterile insects of both the age groups showed similar trends to those of Matin and Hooper (1974) who estimated 89.5% mortality of the adults within 5 weeks of irradiation at 11.5 Krads. Tilton et al. (1966) recorded about 30% mortality in 4 weeks following a dose of 13.2 Krads and Watters and MacQueen (1967) showed that 100% mortality of R. dominica could be achieved 22 weeks after irradiation with a dose of 12.5 Krads. Hassett and Jenkins (1952) achieved 100% mortality in insects 48 days after irradiation with 16,000 r.

3.3.3 Competitiveness between sterile and fertile males

The result is shown by regression analysis for both S. oryzae and R. dominica (Fig. 3.6 and Fig. 3.7). It showed that the complete inhibition of progeny could be achieved with 25 sterile males to 1 fertile male in S. oryzae and 35 sterile males to 1 fertile male in R. dominica even though experimental data showed complete inhibition of progeny production at a ratio of 32 sterile males to 1 fertile male in both S. oryzae and R. dominica. Erdman (1974) reported variable results in both Tribolium confusum and T. castaneum at a male ratio of 20 sterile to 1 fertile. Pradhan et al (1971) showed that a ratio of 200 sterile males

Figure 3.4 Comparative survivals of sterile and fertile males and females of R. dominica (beetles aged 1-7 days when irradiated).

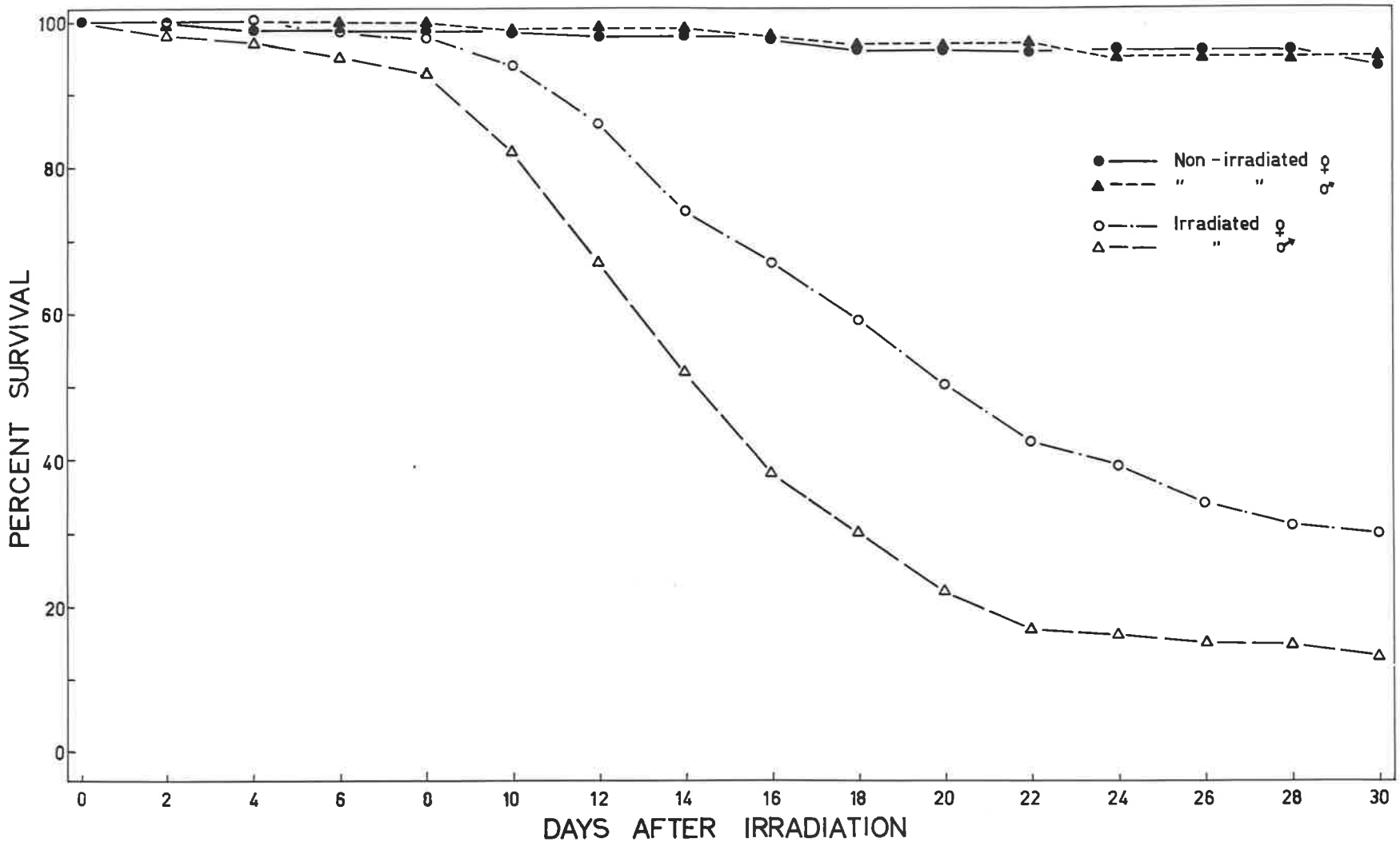

PERCENT SURVIVAL
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
DAYS AFTER IRRADIATION
Non - irradiated ♀
" " ♂
Irradiated ♀
" ♂

Figure 3.5 Comparative survivals of sterile and fertile males and females of R. dominica (beetles aged 8-14 days when irradiated).

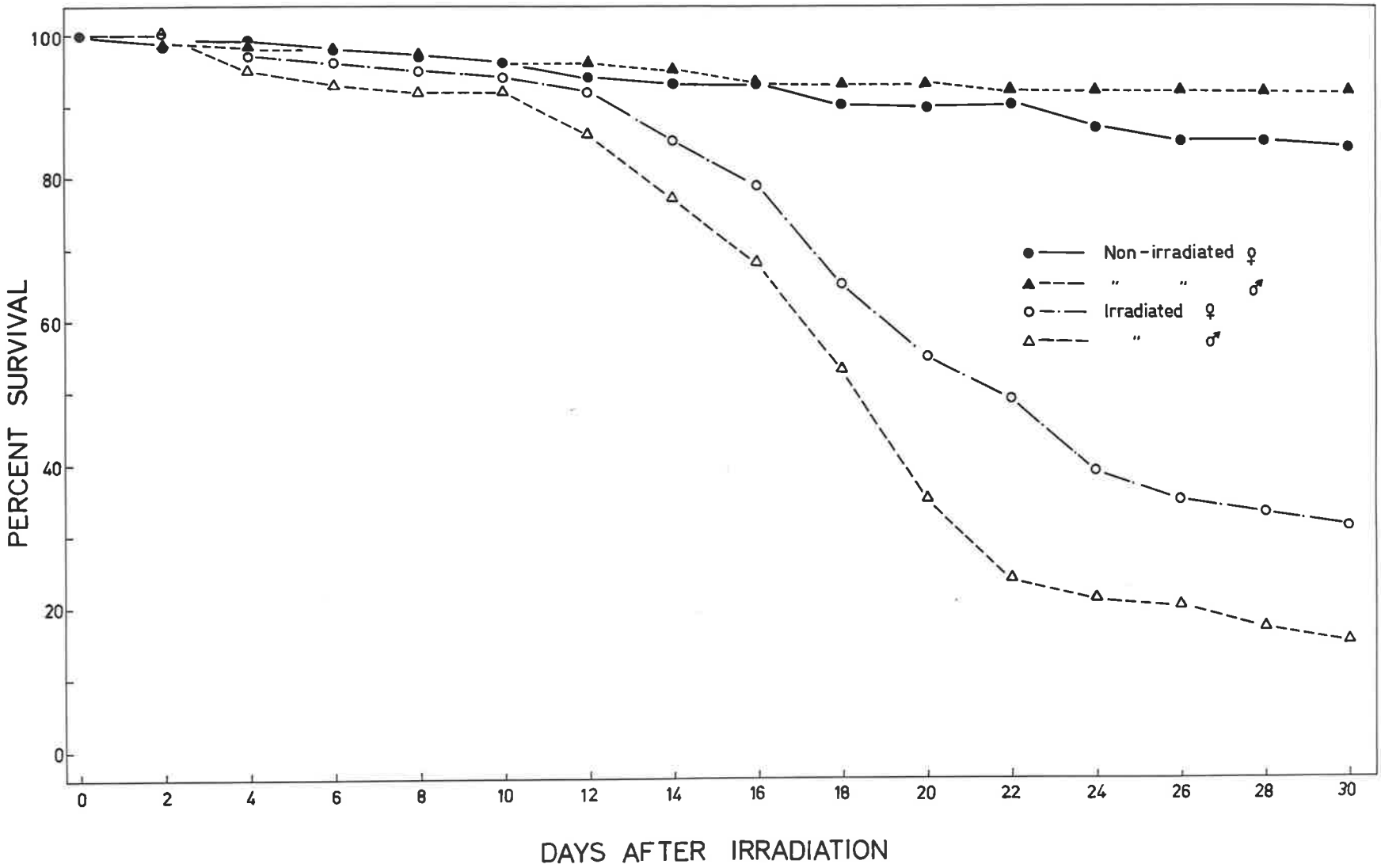

PERCENT SURVIVAL
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
DAYS AFTER IRRADIATION
Non-irradiated ♀
" " ♂
Irradiated ♀
" ♂

Figure 3.6 The regression of the % reduction in progeny on $\log_{10}$ (x + 1) number of sterile to fertile males for S. oryzae.

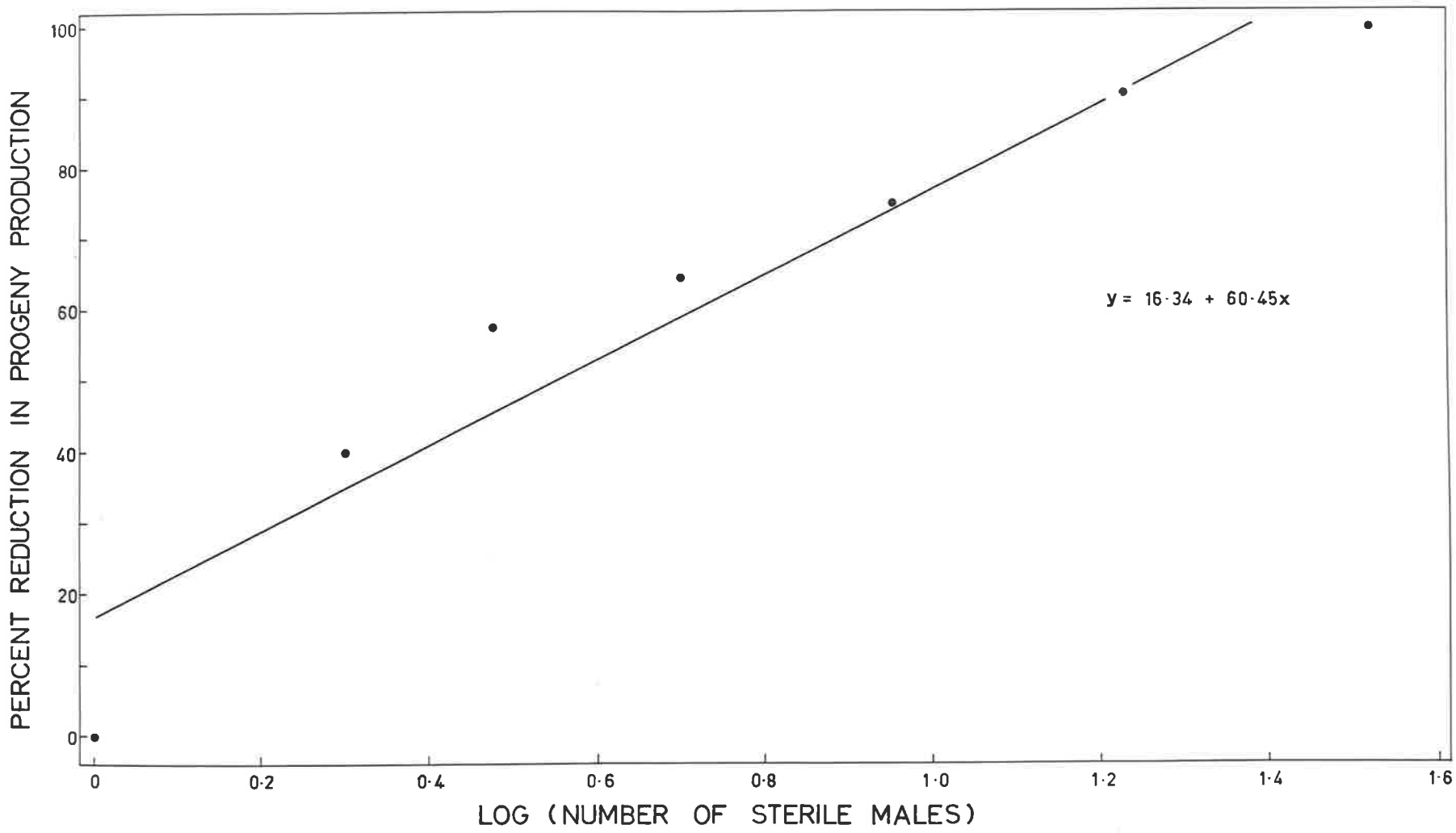
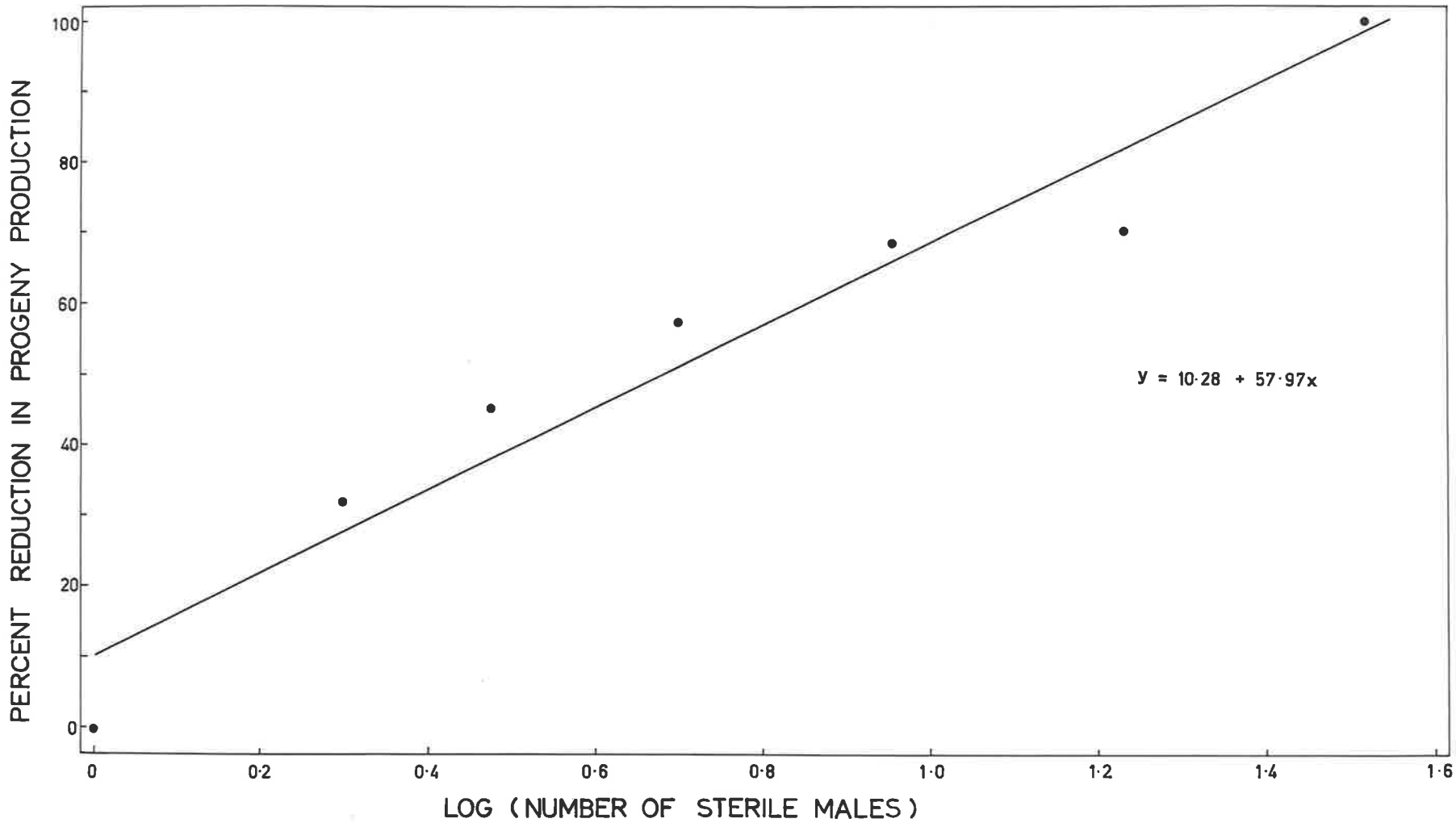

PERCENT REDUCTION IN PROGENY PRODUCTION
100
80
60
40
20
0
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
LOG (NUMBER OF STERILE MALES)
y = 16·34 + 60·45x

Figure 3.7 The regression of the % reduction in progeny on $\log_{10}$ (x + 1) number of sterile to fertile males for R. dominica.


PERCENT REDUCTION IN PROGENY PRODUCTION
100
80
60
40
20
0
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
LOG (NUMBER OF STERILE MALES)
y = 10·28 + 57·97x

to 1 fertile male was required to obtain complete inhibition of population growth in T. castaneum.

3.3.4 Effect of male density on the growth of insect populations

The effect of male density on the population growth for both S. oryzae and R. dominica was illustrated by regression analysis (Fig. 3.8 and Fig. 3.9).

The progeny production in both S. oryzae and R. dominica gradually reduced as the ratio of males to females increased. Total inhibition could not be achieved even at 33 males per female (Fig. 3.8 and Fig. 3.9).

Crombie (1942) showed that as the number of males increases relative to females, fecundity decreases and about 37% reduction is achieved when the sex ratio reaches 15 males to one female in R. dominica.

3.3.5 Conclusion

The results obtained on sterilizing doses and mortalities of the sterilized adults in these experiments are well within the ranges obtained by the other workers mentioned above. The "competitiveness experiments" between sterile and fertile males in both species indicated that the use of the sterile insect release technique in the control of S. oryzae and R. dominica in stored grains was a possibility. The data clearly demonstrates the effect of increasing density of males upon progeny production in both S. oryzae and R. dominica. They suggest that the inhibition of population increase in any sterile insect release method is due to the combined effects of male density and sterility.

Even with the disappointing conclusion of both Crook et al. (1960) and Erdman (1974), the use of sterile insect release method could possibly be used in the control of stored products pests. My results somewhat

Figure 3.8 The regression of the number of progeny produced on $\log_{10}$ (x + 1) number of males to females for *S. oryzae*.

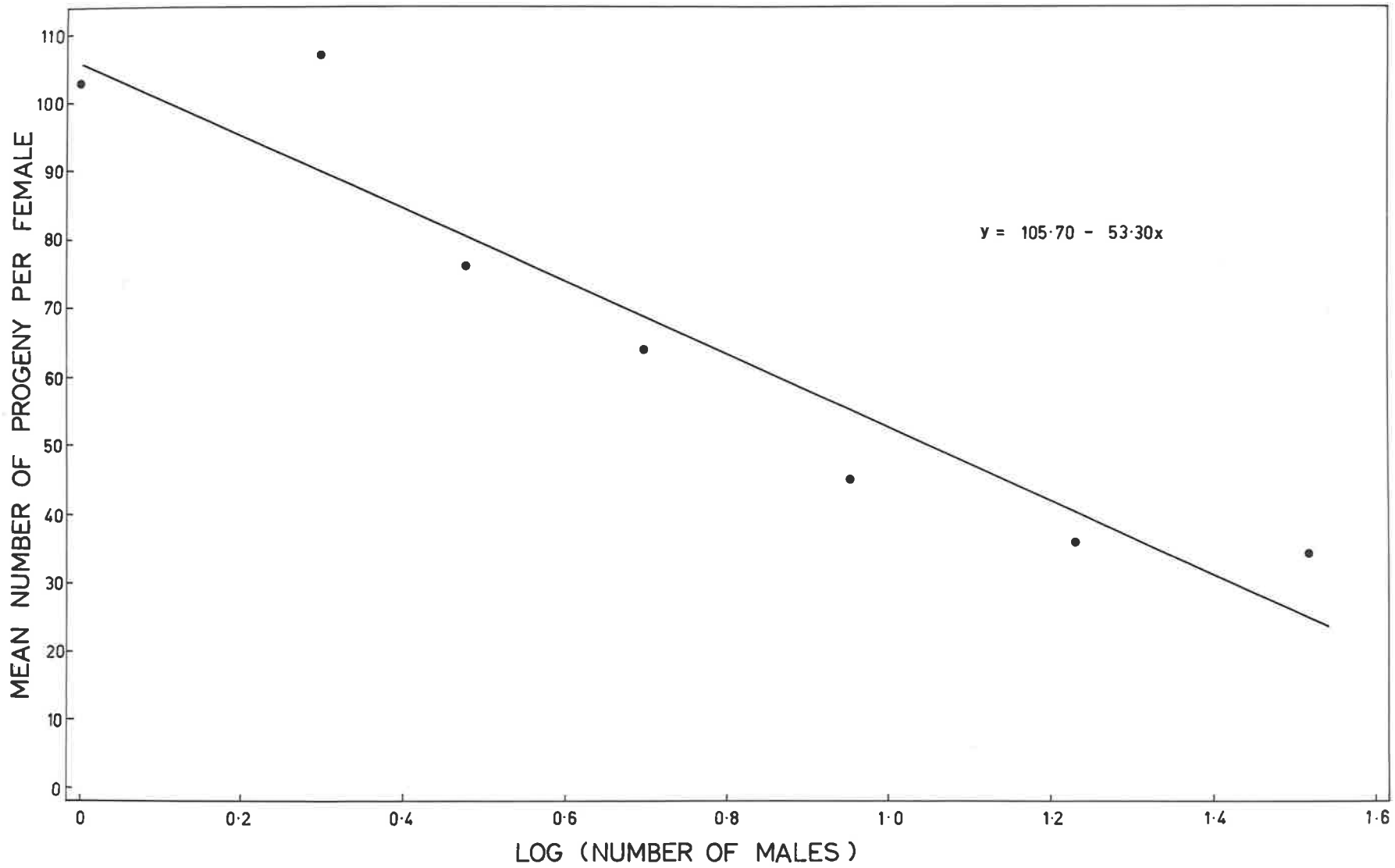
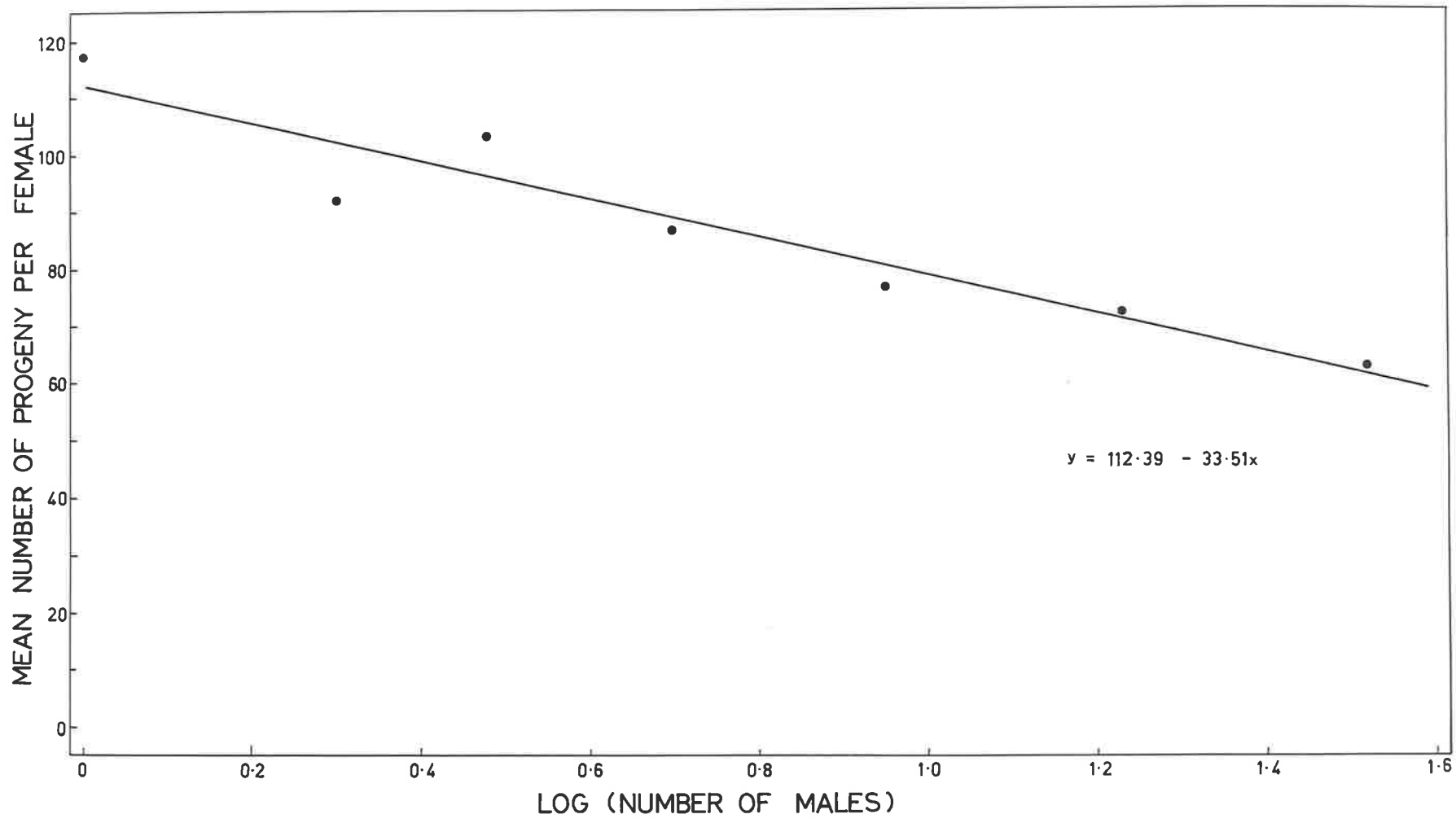

MEAN NUMBER OF PROGENY PER FEMALE
110
100
90
80
70
60
50
40
30
20
10
0
y = 105·70 - 53·30x
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
LOG (NUMBER OF MALES)

Figure 3.9 The regression of the number of progeny produced on $\log_{10}$ (x + 1) number of males to females for R. dominica.


MEAN NUMBER OF PROGENY PER FEMALE
120
100
80
60
40
20
0
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
LOG (NUMBER OF MALES)
y = 112·39 - 33·51x

support the ideas on this subject suggested by Pradhan et al. (1971). I suggest, however, that use of this control technique should be in either of two ways. The first could be based upon assessment of the wild populations and a limited number of releases of a ratio of sterile to fertile males that would inhibit production of offspring. The other would involve periodic releases of lower ratios of sterile to fertile insects over a longer period of time thus causing a more gradual decline in the wild populations.

The factors which militate against using this method in control of grain beetles are concerned with the damage that the sterile insects would do to the stored food and the problems their presence would have upon accepted standards of sanitation and hygiene. Another factor would be the shorter life span of sterile males which would require several releases of sterile males over the period necessary to achieve control of wild populations.

The possible frequent releases of sterile insects due to shorter life span of the sterile males could possibly be minimized either by using sub-sterile insects or by finding other suitable sterilizing agents such as chemosterilants to reduce the rate of mortality caused by irradiation (LaChance et al., 1968).

Another method that could result in overcoming existing disadvantages in the sterile insect release method of insect control, could be induced aggregation of the wild population into a known arena following population suppression with other control methods before applying the sterile male technique. Grain damaged in the arena could then be discarded along with the dead insects or sold for other than human consumption.

4. BEHAVIOURAL STUDIES

4.1 Introduction

The disadvantages of using the sterile insect release method in control of stored products pests were outlined and discussed in Section 3. If the pest could be aggregated into an arena where the control was applied, many of the problems would be overcome because the damage to the grain could be localized, and damaged grain could either be destroyed after the control or could be sold for other than human consumption. This thesis demands control over intraspecific behaviour and, possibly over communication. But it was not known if or how individual beetles in a bulk of grain could detect the presence of other individuals, yet it seemed likely that such communication existed. The basic objective of this section of my studies was to discover whether chemocommunication existed and could be used to manipulate the behaviour of a species. This meant an approach to the understanding of dispersal within simulated natural situations and because of the problems of time and effort in handling the test animals, I choose to restrict the studies to a single species, Sitophilus oryzae.

Measurement of movements of insects with time, particularly in a low density population (a pre-requisite condition for implementing a sterile insect release method), requires a technique that would permit in vitro determinations. The approach using radioisotope tracers and suitable monitoring equipment appeared to have distinct advantages over the successive sampling methods of other workers (Hagstrum and Leach, 1973) and was therefore subsequently developed.

The programme followed a sequence of, firstly, demonstrating the existence of inter-sexual or pheromonic communication in S. oryzae by

monitoring dispersal of the sexes in columns of grain; secondly confirming the inter-sexual responses in a bioassay system; thirdly collecting and utilizing the pheromones in behavioural studies, and fourthly examining the effects of irradiation upon both pheromone production and responses to pheromone.

The latter studies were considered necessary if pheromones and the sterile insect release method were to be integrated in any future control of the rice weevil, S. oryzae.

This section is organized into three sub-sections as a convenient way of explaining their evolution as well as providing the necessary relationship or linkage between them. They are:

4.1.1 A radioisotope technique for studying the dispersal (movement) of S. oryzae in grain.

4.1.2 Studies on dispersal (movement) of S. oryzae adults in low density populations in grain.

4.1.3 Demonstration, collection, isolation and bioassay of pheromones of S. oryzae.

4.1.1 A radioisotope technique for studying the dispersal (movement) of S. oryzae in grain.

4.1.11 Introduction

Studies on the dispersal or movement of delitescent insects in their natural or experimental environments are usually carried out by periodic sampling of habitats (Birch, 1946; Howe, 1951; Surtees, 1964b; Ogden, 1970; Hagstrum and Leach, 1973) or by erecting artificial arenas where visual sequential records of the position of the insects may be recorded (Surtees, 1963a) or by using special dispersal apparatus (Naylor,

1959, 1965; Tschinkel and Belle 1976). The first of these techniques requires handling and identification of the insects which places test animals at risk of both damage and physiological disturbance. The second method, though obviating handling of the insects, uses a "sandwich technique" where the habitat is just two-grain diameters deep. This introduces a stratification of the habitat perhaps only comparable with the surface layers of grain in usual storage containers. The third method has some characteristics of the other two. It also eliminates the need to handle the insects but it is necessary to identify them. The habitat (arena) is also only a few cm thick, again comparable with the surface layer of grain in usual storage facilities.

The technique chosen uses the short lived radioisotope Scandium-46 as the labelling material, was designed to measure movements of individual insects, and, as such, had different objectives from those of other workers mentioned above. The design was appropriate to analyses of populations at very low density, where intraspecific or inter-sexual communication is vital for establishment and reproduction of a species. Scandium-46 was attached to an insect, which was confined within a column of grain that permitted detection of the isotope no matter what position in the column the insect occupied.

Radioisotopes have been attached to insects using metal foils of RaSO4 (Tomes and Brian, 1946; Brian, 1947), ^{60}CO in the form of wires (Arnason et al., 1950; Fuller et al., 1951; Fredericksen and Lilly, 1955; Godfrey, 1954), or in paints (Sullivan, 1953; Green et al., 1957), ^{182}Ta in wires (Banks, 1955; Banks et al., 1961) and ^{46}Sc in paints (Davis and Nagel, 1956; Godwin et al., 1957; Lamb et al., 1971). In most such experiments the insects were labelled for detection on plants or in the ground. In stored products pests such as Sitophilus zeamais, ^{86}Rb has been

used to study its flight behaviour (Giles, 1969).

4.1.12 Labelling of the insects

Rice weevil adults were obtained (if not otherwise stated) from a laboratory culture maintained at 30 ± 0.5°C and at 75% relative humidity in wheat (Heron) of 14.5% moisture content. Insects of a particular age and sex were obtained by sieving the stock culture as described in Section 2.

Scandium-46 emitting β-particles (0.36 Mev) and γ-rays (0.89 and 1.12 Mev) and has a half-life of 85 days (Lamb et al., 1971) was obtained from the Australian Atomic Energy Commission as the oxide in acetyl acetonate in petroleum ether solution. Batches varied in activity between 58 and 110 µCi per 200 µl.

The labelling material was prepared by diluting 50 µl of the scandium-46 solution with 100 µl of dry acetone and then adding three drops (approx. 100 µl) of nail varnish. The resulting paint contained 0.058 - 0.11 µCi ^{46}Sc μl^{-1}. The paint was taken up into a microsyringe fitted with a manipulator delivering a minimum volume of 0.2 µl.

Before applying the paint, the beetle was immobilized by chilling it in a petri dish on ices. It was then held by its rostrum in a groove of plasticene in such a way that the elytron was exposed. The required amount of paint was then applied to the elytron and the insect held for 2-3 minutes for the paint to dry. The insect was then checked for damage or restriction of its movement due to overflow of paint to other parts of the body. Correctly marked, undamaged, active insects were retained, all others were destroyed.

Because the measurable activity of the marker varied between insects, test animals for each experiment were further selected so that

variation in activity across the group did not exceed 20% from the mean.

Two preliminary experiments were carried out to check whether the presence of the radioactive paint had any effect on the mobility and life span of test weevils. In each experiment, five treatments were considered, two with radioactive paint, 0.5 and 1.0 µl per insect containing 0.046 and 0.092 µCi ^{46}Sc, two with 0.5 and 1.0 µl of non-radioactive paint and one without paint.

Mobility: Mobility was tested using 1-2 day-old beetles. Insects were allowed to walk individually up a thin metal rod, 25 cm long, inclined at 60^{o} to the horizontal where climbing ability of S. oryzae is not affected (Cline and Highland, 1976). The time taken for each insect to climb the rod was recorded successively for the same period (9.00 - 11.00 a.m.) on alternate days for two weeks. Initially there were six insects in each treatment but damage reduced the number in some treatments to four at the last test. The mean climbing time taken by insects in each treatment is given in Table 4.1.

An analysis of variance (treatment x time) showed no significant differences between the treatments or times (Table 4.2).

Longevity: Insects, 1-2 day-old, some with radioactive paint, others with non-radioactive paint and some others unpainted, were held in clean test wheat in standard rearing containers for two weeks at 30 $\pm$ $0.5^{o}C$ and 75% relative humidity. The mortality of the insects was recorded every day for two weeks.

Of the 100 insects tested only one insect in the unpainted treatment died within the experimental period.

As a result of the experiments on mobility and longevity, it was concluded that ^{46}Sc could confidently be used as a marker in investigations

TABLE 4.1 Time (seconds) taken for adults of S. oryzae to climb an inclined rod. Data as means ± S.E. for 4 to 6 replicates per treatment per day.

Treatments	Time Taken		
	Day-0	Day-8	Day-14
unmarked	124.66 ± 5.10	108.40 ± 15.40	189.80 ± 29.74
0.5 µl non-radioactive paint	101.60 ± 10.82	91.00 ± 18.66	125.00 ± 15.88
1.0 µl non-radioactive paint	110.66 ± 13.45	99.40 ± 12.59	139.00 ± 43.21
0.5 µl radio-active paint	118.16 ± 10.30	101.00 ± 12.51	113.60 ± 8.95
1.0 µl radio-active paint	140.00 ± 17.06	103.80 ± 16.36	133.60 ± 16.63

TABLE 4.2 Analysis of variance for the effect of ^{46}Sc on the mobility of S. oryzae in time (2 weeks).

Source	df	SS	MS	F	P
Times	7	187769	31294	1.92	N.S.
Treatments	4	78027	19506	1.19	N.S.
Times x Treatments	28	292302	10439	0.64	N.S.
Error	170	2768243	16283		
Total	209	3326342			

extending up to two weeks, without measurable effects from the radioisotope at the dosages tested.

4.1.13 Experimental columns of grain

Two sizes of P.V.C. tubes were used, 30 cm long, 2.5 cm diameter and 60 cm long, 4.5 cm diameter with a wall 2 mm thick, through which γ-rays (but not β-particles) could be detected. The tubes were filled with the standard wheat to simulate a column such as is found in most modern silos.

A collimated γ-scintillation counter was used to detect the presence of marked insects inside the experimental column of grain.

Each column was held vertically, clamped in position against a graduated scale. The counter head, lined up at a fixed distance of 2 mm from the column, could be moved vertically to any position with reference to the scale.

4.1.14 Calibration of column

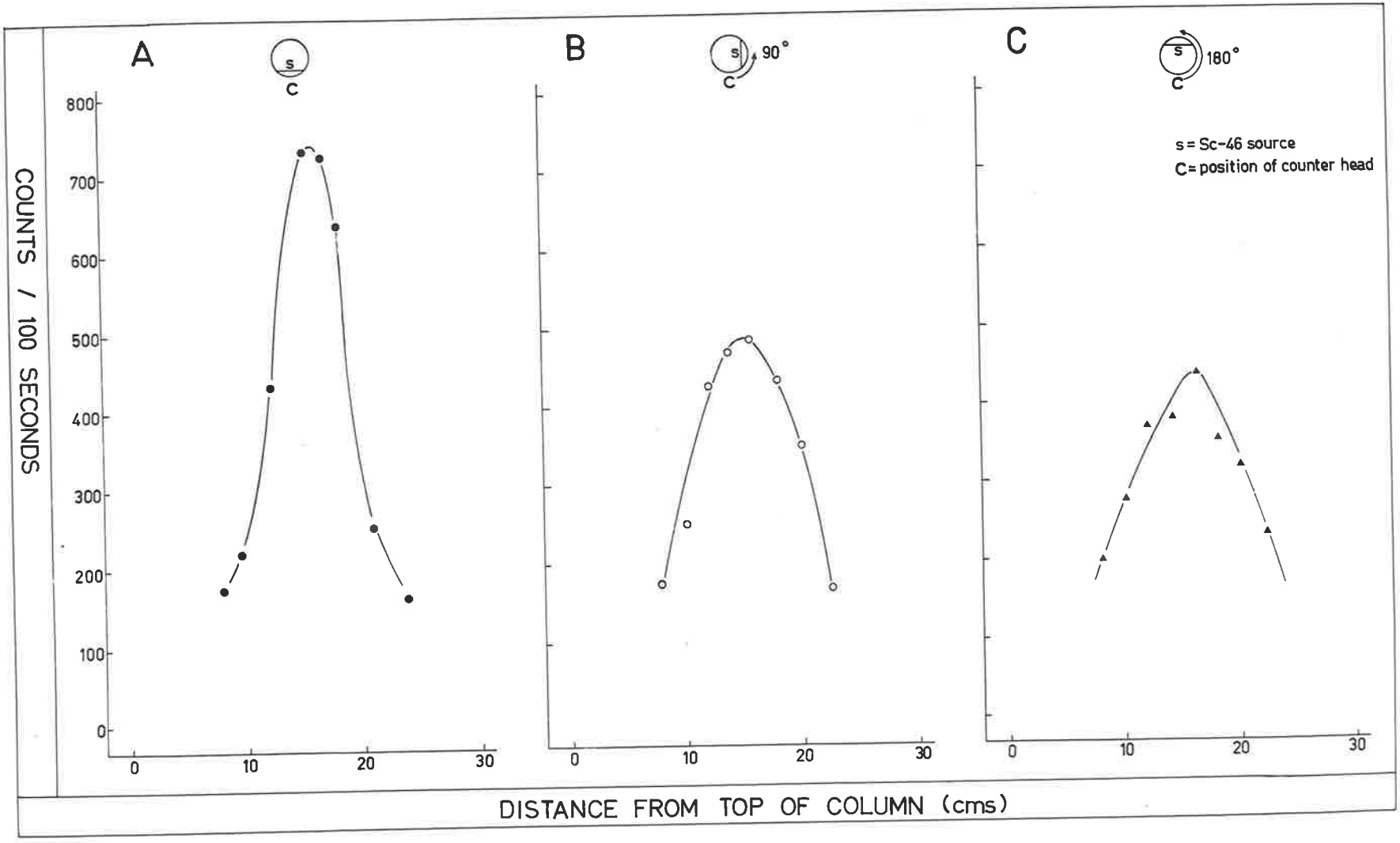
A disc of plastic to which a drop of ^{46}SC paint had been applied, was placed within the 30 cm column against the wall nearest to the counter, at a distance of 15 cm from the end of the column. Readings were taken of the count rate (counts 100 sec^{-1}) at intervals along the length of the column. The result is plotted in Fig. 4.1A. The column was then rotated, first through 90^{o}, then through 180^{o} and the counts repeated at each position (Fig. 4.1B and 4.1C respectively). Taking the background count rate at 175 counts 100 sec^{-1}, the width of the curves at half maximum height vary from 7.0 to 10.0 cm and the height varies from the maximum value to one half this value according to whether the source is at the near or far side of the column.

Figure 4.1 Calibration of column of wheat with a known source of ^{46}Sc.

A. Counter head at 0^{o} position.

B. Counter head at 90^{o} position.

C. Counter head at 180^{o} position.


A
B
C
90°
180°
s
C
s = Sc-46 source
C = position of counter head
COUNTS / 100 SECONDS
DISTANCE FROM TOP OF COLUMN (cms)
0
100
200
300
400
500
600
700
800
10
20
30

With two sources of radioactivity present in the column, the count rate/distance plot consists of a combination of two curves of the form shown in Fig. 4.1A, B and C having parameters within the limits given above. Figure 4.1D, E, F and G show the resultant theoretical curves obtained when two individual curves are combined, one representing the situation where the radioactive source is at the near side of the column (0^{o}) and the other when the source is to the side (90^{o}). The separation between the sources is 0, 2.5, 5.0 and 7.5 cm respectively. It was concluded that in the practical situation it would not be possible to separate the two sources when the sources were less than 2.5 cm apart. With careful measurement of the count rate of the source in an accurately known initial position, it should also be possible to determine its horizontal position relative to the counter; that is whether the source is at the near or far side of the column.

4.1.15 Detection of live labelled weevils in a column of grain

Experiments were designed to follow the position of a pair of 1-2 day-old, ^{46}SC marked, adult rice weevils in a 60 cm column of test whest at 25 $\pm$ 0.5^{o}C and 70% relative humidity. The individuals obtained from culture maintained at 25 $\pm$ 0.5^{o}C and 75% relative humidity were chilled and introduced into the column 15 cm from either end, the column was provided with covers: gauze (bottom and muslin (top). Positions of beetles were determined daily. Some of the results from two of the sources are shown as count rate/distance plots in Fig. 4.2.

The positions of the insects clearly indicated that their separation decreases over a period of days, the two insects coming to within

Figure 4.1 D, E, F and G showing resultant theoretical curves obtained when the radioisotope is at different positions relative to the counter head (see text).

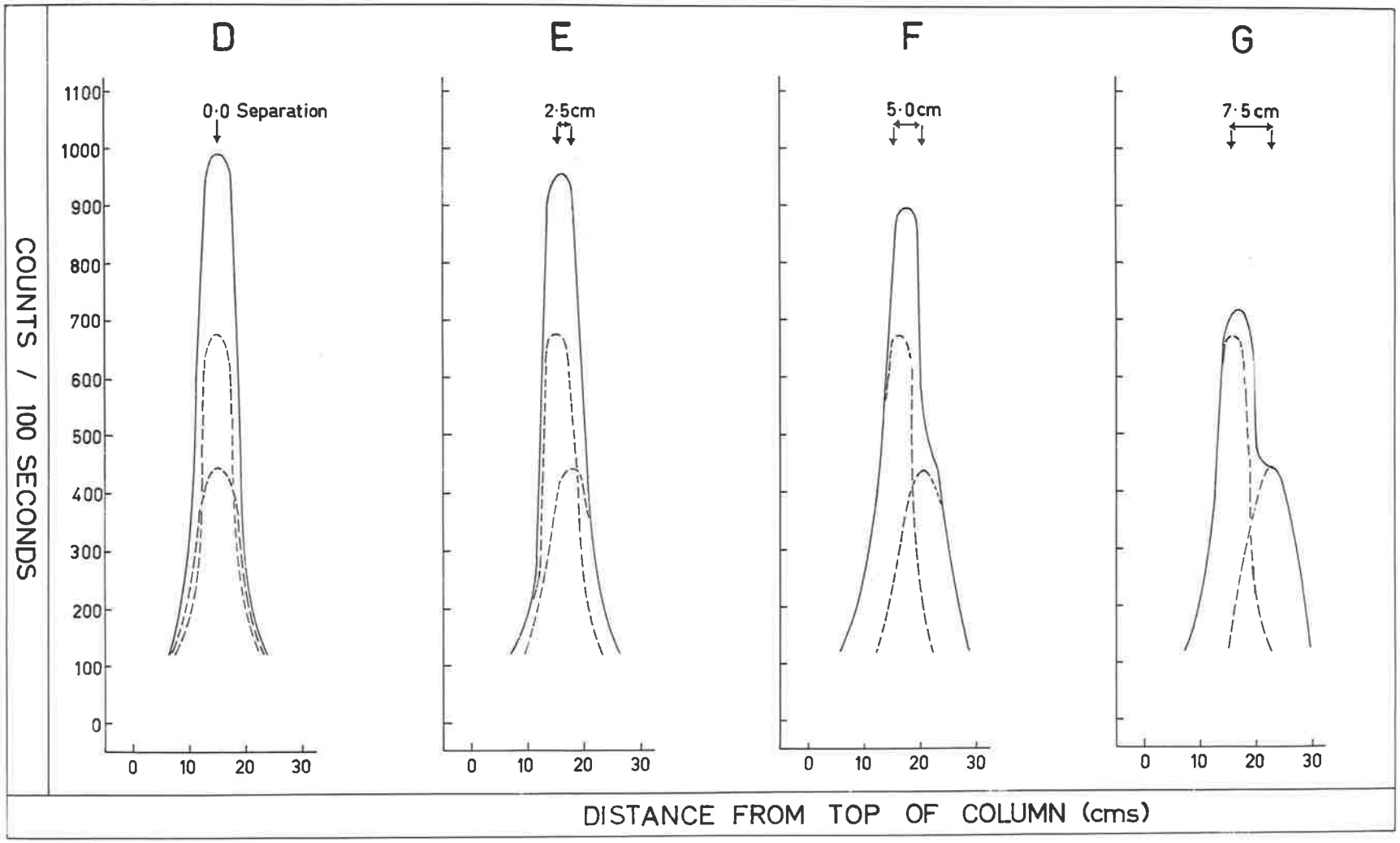
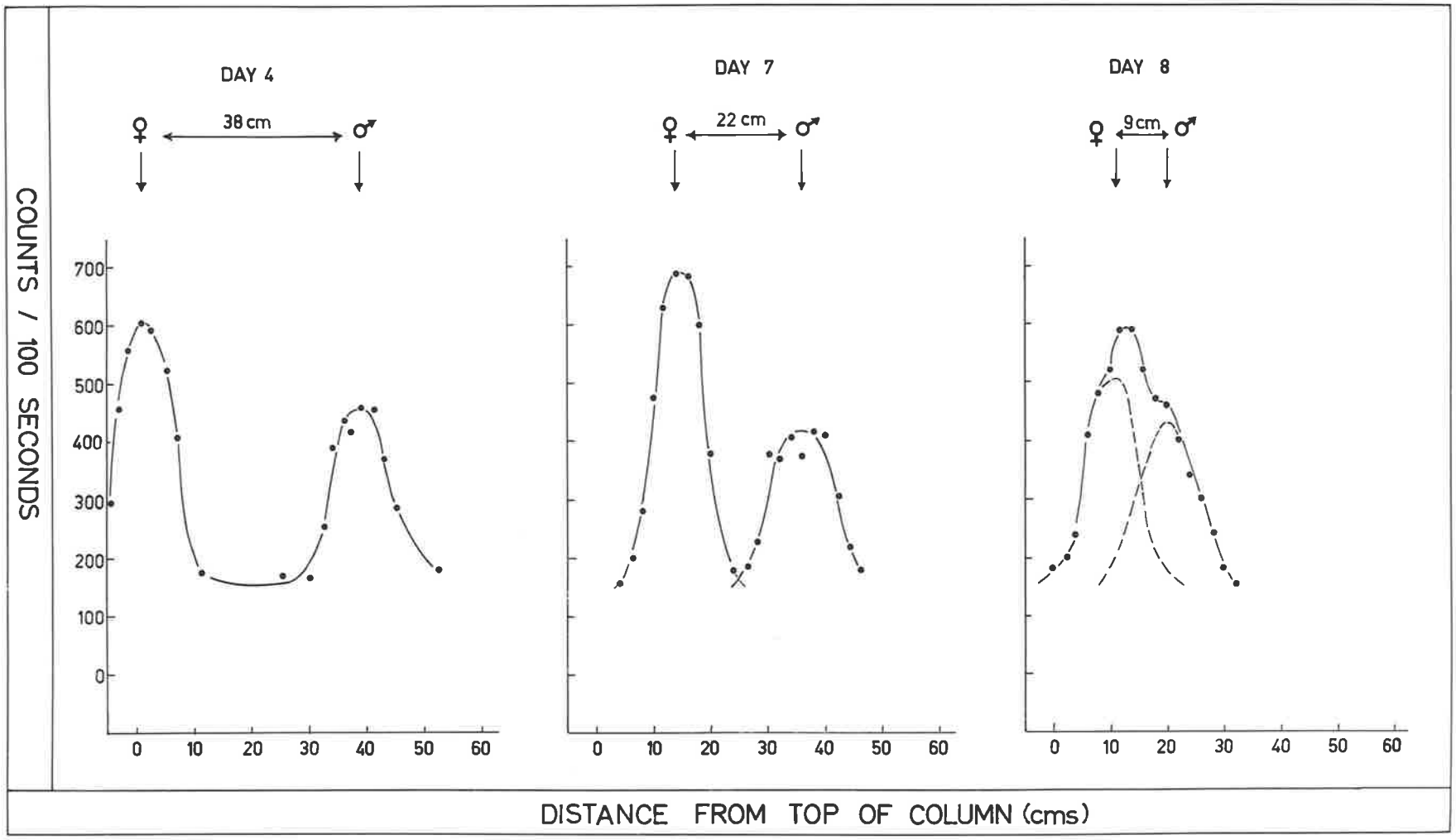

D
E
F
G
0·0 Separation
2·5cm
5·0cm
7·5cm
COUNTS / 100 SECONDS
1100
1000
900
800
700
600
500
400
300
200
100
0
0
10
20
30
DISTANCE FROM TOP OF COLUMN (cms)

Figure 4.2 Detection of marked weevils in a column of grain.


DAY 4
38 cm
DAY 7
22 cm
DAY 8
9 cm
COUNTS / 100 SECONDS
700
600
500
400
300
200
100
0
0 10 20 30 40 50 60
DISTANCE FROM TOP OF COLUMN (cms)

9 cm of each other in 8 days.

The evidence presented clearly demonstrates the usefulness of radioisotope tracers in studies of the movement of individual rice weevils in stored grain. It has limitations due to the numbers of insects that can be used, the time taken to record results and the least distance at which an accurate measure of the location of the labelled insect can be detected. Nevertheless, for studies of intra-specific communication between insects in grain, the technique described here has given some useful results.

4.1.2 Studies on dispersal (movement) of S. oryzae adults in low density populations in grain

4.1.21 Introduction

Movements of grain beetles do occur in grain and this movement may be influenced by many factors such as temperature, moisture content and sizes of grain, density of adults or larvae, condition of the food, the sex of insects and whether they have mated or are unmated (Birch, 1946; Howe, 1951; Surtees, 1963a, b, c, 1964a, b; Naylor, 1959, 1961, 1965; Ghent, 1963, 1966; Ogden, 1970; Hagstrum and Leach, 1973; Tschinkel and Belle, 1976). We know little about the influence the individual insect exerts upon such dispersal. Naturally enough, insect to insect stimuli may control certain types of dispersal and this may be brought about in several ways.

The objective of this study was therefore centred upon the influence of sex upon the attraction or repellence of insects within columns of grain to determine whether insect-produced signals were part of the dispersal process.

4.1.22 Procedures and Results

The movements of individual insects were observed in 30 cm and 60 cm columns of grain. Two adult unmated weevils, were used for each experiment. Each experiment was duplicated and they were carried out at the same time and referred to as experiment I and II. The temperature and relative humidity for all experiments in this section were 25 $\pm$ 0.5^{o}C and 70% respectively.

Dispersal in 30 cm columns: The experimental pair of weevils, 1-2 day-old, were marked one with 0.5 µl and the other with 1.0 µl of ^{46}SC paint and were selected for individual tests within a radioisotope activity variation of $\pm$ 20% from the mean.

The marked insects so chosen were then chilled and introduced into the column of wheat so that one was located 10 cm from each end. No anaesthetic was used because of the chance that it would impair behaviour (Brady and Smithwick, 1968). Columns were positioned vertically in the controlled environmental cabinet and insects were allowed to settle for 24 hours and then their positions were monitored daily between 10 a.m. and 4 p.m. for three days.

Three combinations of sexes were tested for movements. They were: ♂/♀, ♂/♂ and ♀/♀ and the experiments were referred to as A, B and C.

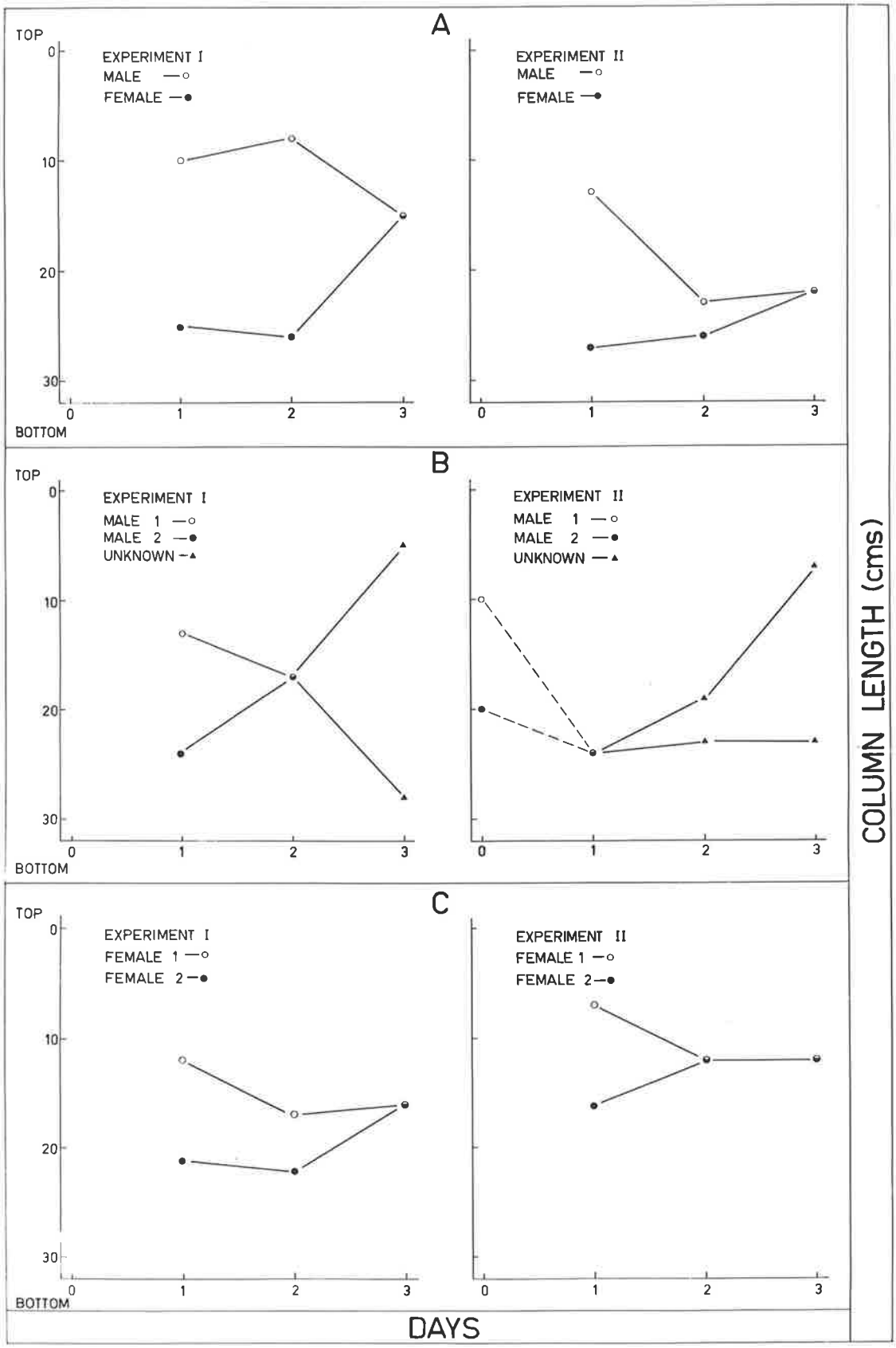
The movements of individuals provided consistent evidence that beetles 10 cm apart in grain approached close to one another within 3 days irrespective of the sexes present. Opposite sexes appeared to meet on about day 3, male/male combinations on day 1 or day 2 and female/female couples on day 2 or day 3. Following the meeting the individuals usually separated with the insect initially placed above the other moving upward and the other downward (Fig. 4.3A, B and C and duplicates).

Figure 4.3 Sequential daily positions of insects in 30 cm columns of grain as detected by radioisotope monitoring.

AI and AII. Both sexes present ♂ above ♀.

BI and BII. Males only present.

CI and CII. Females only present.


A
TOP
0
10
20
30
BOTTOM
EXPERIMENT I
MALE —○
FEMALE —●
EXPERIMENT II
MALE —○
FEMALE —●
0
1
2
3
B
TOP
BOTTOM
EXPERIMENT I
MALE 1 —○
MALE 2 —●
UNKNOWN —▲
EXPERIMENT II
MALE 1 —○
MALE 2 —●
UNKNOWN —▲
C
TOP
BOTTOM
EXPERIMENT I
FEMALE 1 —○
FEMALE 2—●
EXPERIMENT II
FEMALE 1 —○
FEMALE 2—●
DAYS
COLUMN LENGTH (cms)

This consistent behaviour suggested that beetles were responding to signals from their partners, that the response was comparatively rapid and that attraction was followed by a period of non-attraction or repulsion. The design of the experiment was not sensitive enough to be able to distinguish whether the signals were pheromonic or aural because the beetles were too close at the start and approached one another too quickly.

Dispersal in 60 cm columns: Because of the short comings due to the short distance between the insects in 30 cm columns, experiments were designed in relatively bigger 60 cm columns. The methods used were similar to those in the 30 cm columns except that each insect was introduced 15 cm from either end and the experiments continued for a maximum period of 10 days.

The combination of sexes tested were: ♂/♂, ♂/♀, ♀/♂ and ♀/♀ with the first of the pair above the other and they are referred to as experiment A, B, C and D.

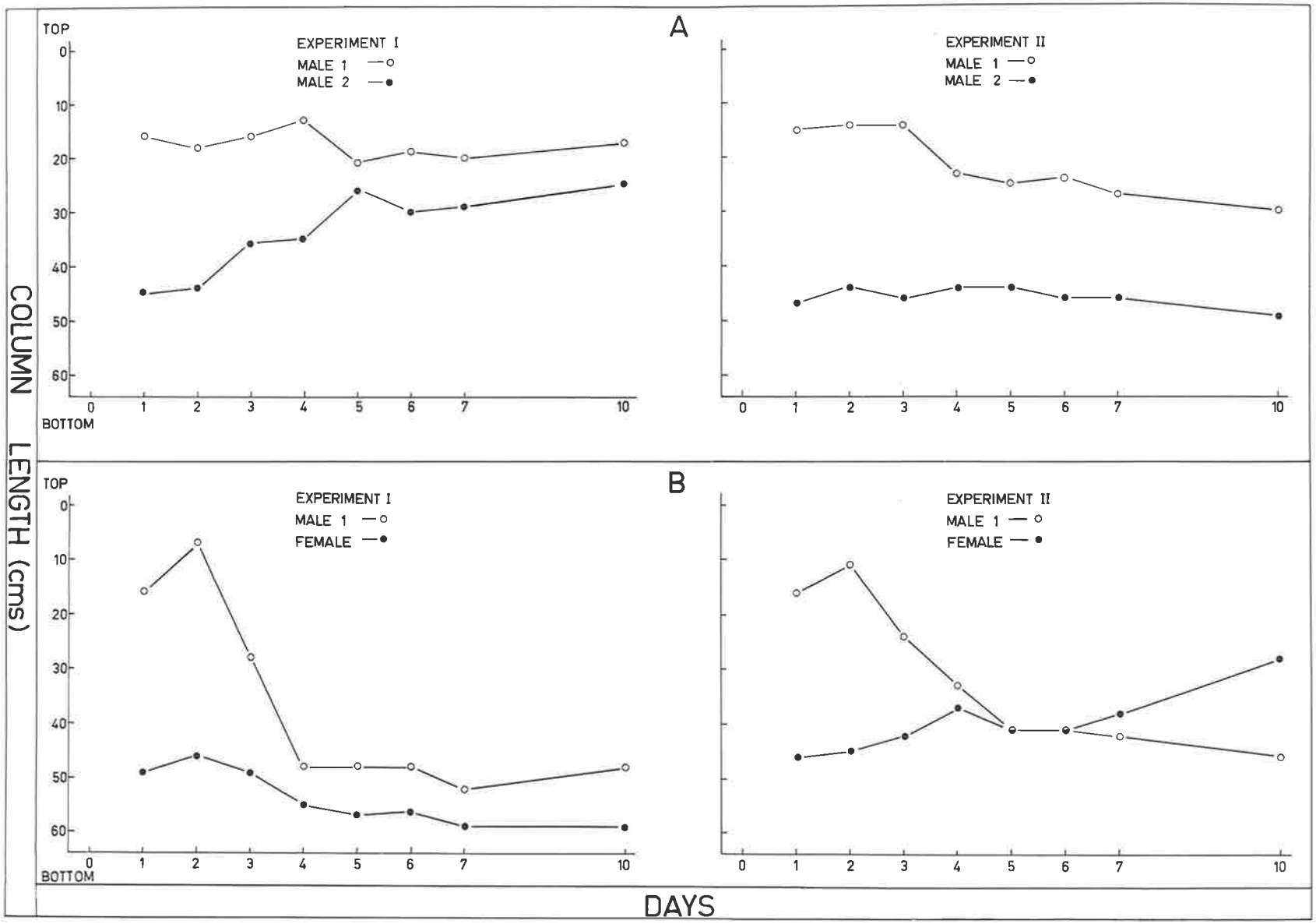
As the initial distance of separation may effect intraspecific communication, the experiments in the 60 cm columns of grain were completed with test animals 30 cm apart or 3 times the distance of the first test in the 30 cm columns. The male/male response was not as marked as in the earlier experiments with an indication of approach to each other by about day 5 and with maintenance of a relatively consistent distance between them thereafter (Fig. 4.4 AI and II). Where opposite sexes were concerned the male changed direction toward the female on day 2 and appeared to meet her about 2 days later. Thereafter they separated but the male did not always move back in the direction from which he came (Fig. 4.4 BI and II).

When females were located above males at the commencement of the test, the individuals did not appear to meet within the 10 day experimental

Figure 4.4 Sequential daily positions of insects in 60 cm columns of grain as detected by radioisotope monitoring.

AI and AII. Males only present.

BI and BII. Both sexes present ♂ above ♀.


A
B
TOP
BOTTOM
EXPERIMENT I
MALE 1
MALE 2
EXPERIMENT II
MALE 1
MALE 2
FEMALE
0
10
20
30
40
50
60
0
1
2
3
4
5
6
7
10
COLUMN LENGTH (cms)
DAYS

period. Both tended to move slightly downward in the columns (Fig. 4.4 CI and II). The female/female tests did not indicate attraction of individuals toward each other (Fig. 4.4 DI and II). These tests indicated that initial distance apart was important to intraspecific communication. As the opposite sexes did appear to meet while other combinations failed, a female/male signal seemed to operate but only when the male was above the female. While the evidence was not absolutely clear, I felt that sound in a column of this kind ought to go in both directions equally due to a possible "Tunnel effect". A volatile chemical would most likely rise in a vertical tunnel, particularly as a column of grain tends to increase in temperature deep in the grain and to have its lowest temperature at the surface. None of these characteristics were tested but, in the next series of experiments, a measured airflow was provided in the columns to further test the direction from which a signal (stimulus) was received.

Dispersal in 60 cm columns with airflow: A glass tube was inserted through a rubber stopper at the bottom of the column and connected to a suction pump through a flow meter. Air was drawn through the column at a rate of 1.5 l min^{-1}. The combinations of sexes tested were ♀/♂ and ♂/♀.

All other methods used were similar to those in the previous experiments in 60 cm columns.

The results clearly demonstrated that a strong attraction of ♂ to ♀ existed from about day 4 with contact of the sexes between day 5 and 8. This was clearly apparent when the airflow was from ♀ to ♂ (Fig. 4.5 AI and II). A similar direct attraction of ♀ to ♂ was recorded in experiment 4.5 BII. The results were encouraging to the developing hypothesis that a pheromonic system of intraspecific communication existed in S. oryzae.

Dispersal and interactions of sterile/fertile insects in 60 cm colums with

Figure 4.4 Sequential daily positions of insects in 60 cm columns of grain as detected by radioisotope monitoring.

CI and CII. Both sexes present ♀ above ♂.

DI and DII. Females only present.

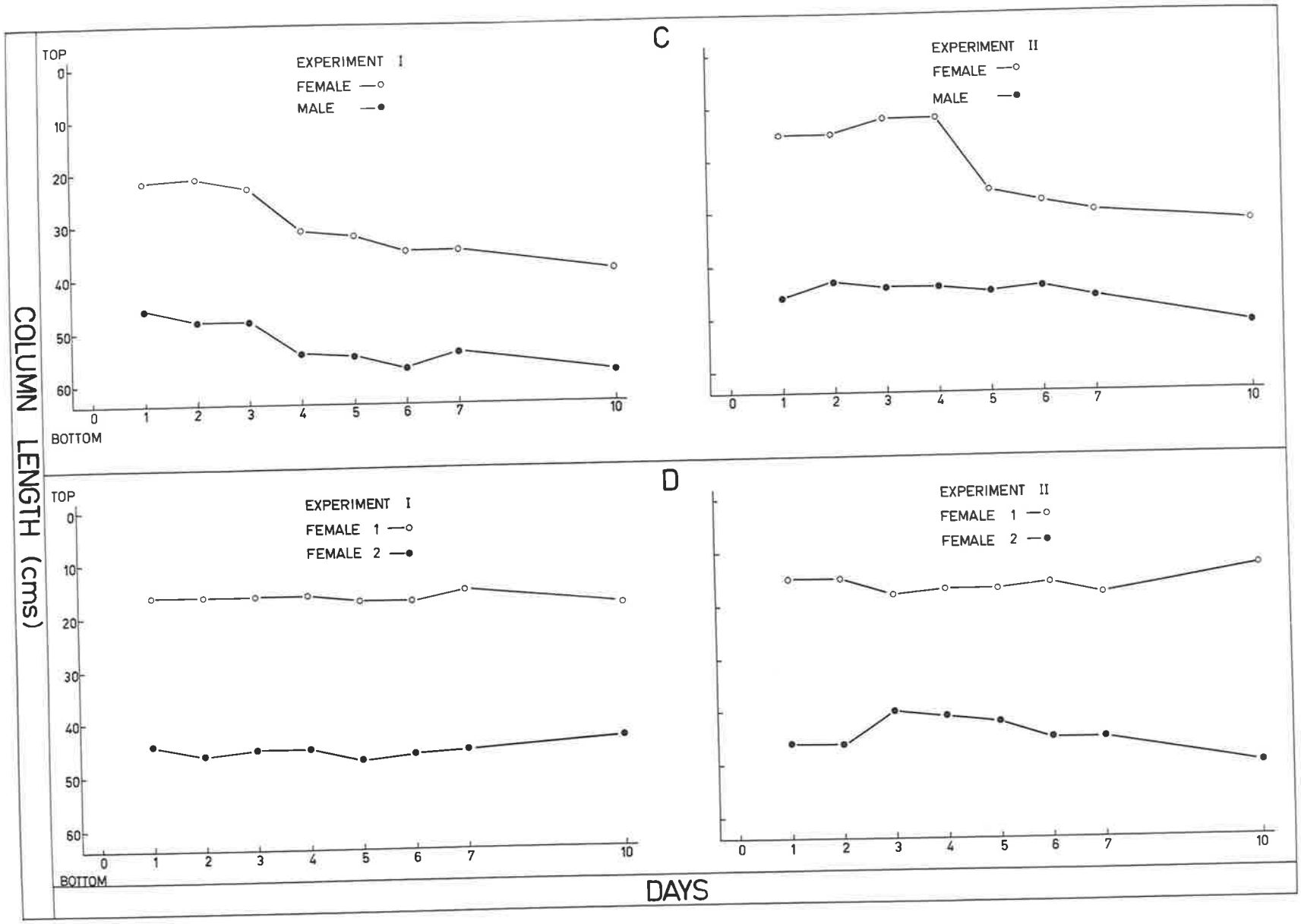
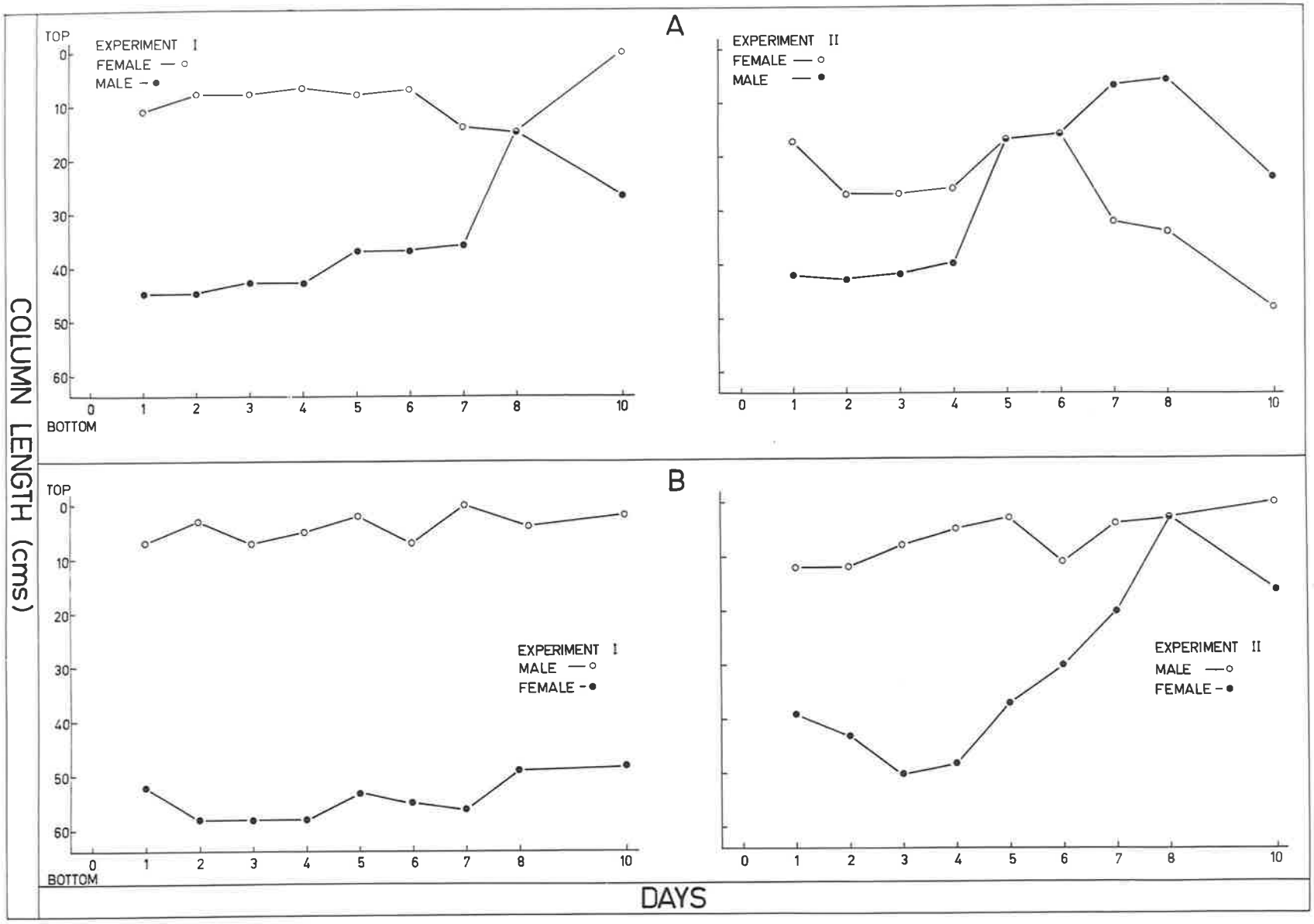

C
EXPERIMENT I
FEMALE
MALE
TOP
BOTTOM
EXPERIMENT II
FEMALE
MALE
D
EXPERIMENT I
FEMALE 1
FEMALE 2
EXPERIMENT II
FEMALE 1
FEMALE 2
COLUMN LENGTH (cms)
DAYS

Figure 4.5 Sequentialy daily positions of insects in 60 cm columns of grain as detected by radioisotope monitoring (specific air-flow provided down each column).

AI and AII. Both sexes present ♀ above ♂.

BI and BII. Both sexes present ♂ above ♀.


A
B
TOP
BOTTOM
EXPERIMENT I
FEMALE
MALE
EXPERIMENT II
FEMALE
MALE
EXPERIMENT I
MALE
FEMALE
EXPERIMENT II
MALE
FEMALE
0
10
20
30
40
50
60
1
2
3
4
5
6
7
8
10
COLUMN LENGTH (cms)
DAYS

air flow: A vital part of the hypothesis was to determine the effect of irradiation sterility upon intraspecific communication and therefore another series of experiments were conducted in 60 cm columns with air flows where dispersal of sterile and fertile individuals were examined to provide required evidence. All materials and methods were as described for previous experiments.

The combinations of sexes and sterile (irradiated = S) and fertile (non-irradiated = F) insects tested were: F♀/S♂, S♀/F♂ and S♀/S♂ and the experiments were referred to as A, B and C respectively.

The results showed that:

(i) Fertile ♀ did not attract sterile ♂ (Fig. 4.6 AI and II).

(ii) Sterile ♀ attracted fertile ♂ (Fig. 4.6 BI and II).

(iii) Sterile ♀ did not attract sterile ♂ (Fig. 4.6 CI and II).

In all cases the air flow was from ♀ toward ♂, and all results were based upon an experimental period of 10 days.

4.1.23 Discussion

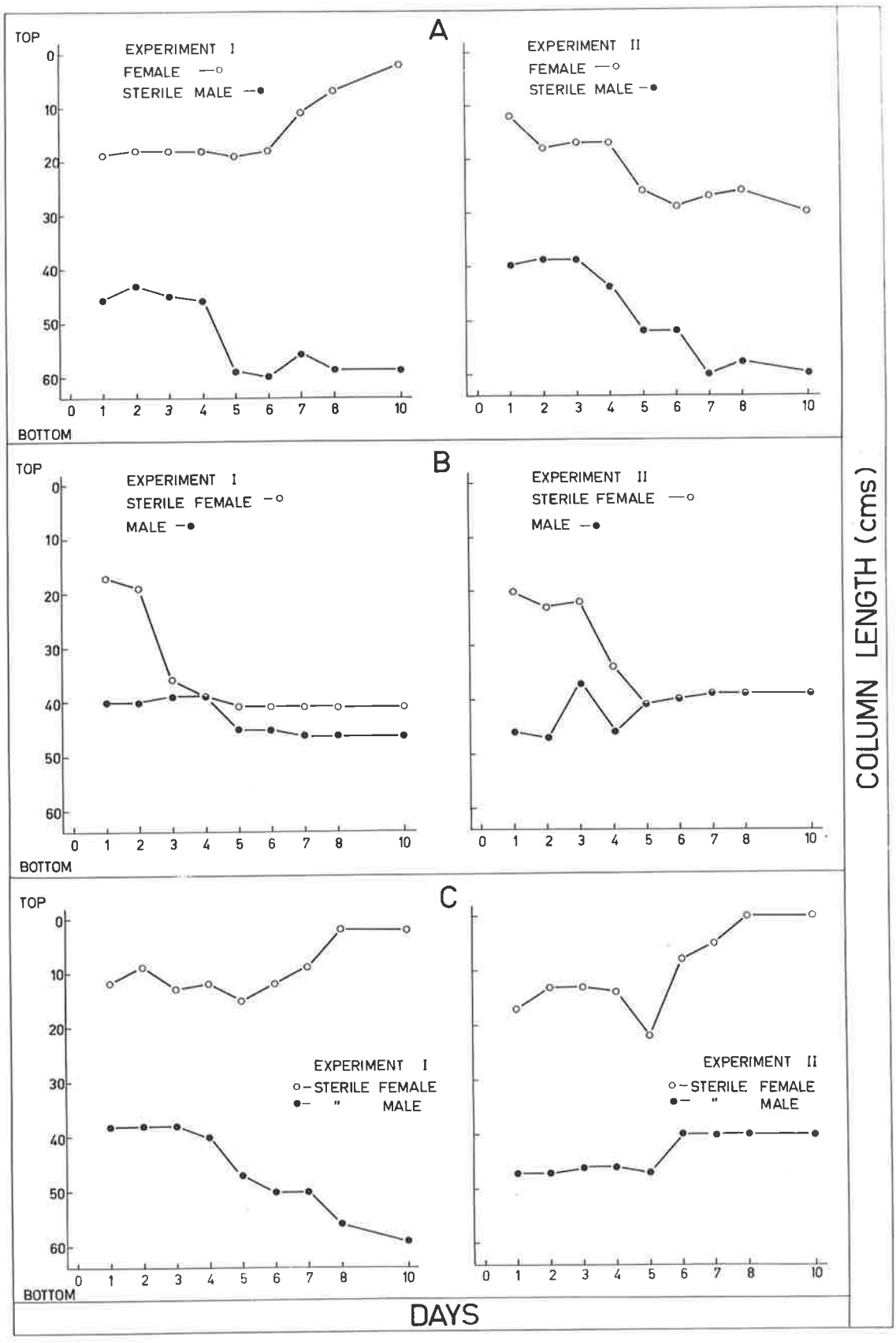
Isolated individuals of Sitophilus oryzae and Tribolium castaneum appear to react in a similar way to the initial movements recorded here for S. oryzae, when they are maintained in similar conditions of temperature and grain moisture content (Surtees, 1963a, c) to those used in the above experiments. Ogden (1970) reported the movements of isolated sexes of Tribolium castaneum and Tribolium confusum and Hagstrum and Leach (1973) on Tribolium castaneum. Neither study attempted to determine the interaction between the sexes as my experiments did. This data demonstrated the possibility of a sex pheromone in S. oryzae; indicated that the main attractive force was produced by the female (with possibly one also produced by males); and showed that male response was adversely affected by

Figure 4.6 Sequential daily positions of insects (sterile and fertile) in 60 cm columns of grain as detected by radioisotope monitoring (specific air-flow provided down each column).

AI and AII. Both sexes present fertile ♀ above sterile ♂.

BI and BII. Both sexes present sterile ♀ above fertile ♂.

CI and CII. Both sexes present sterile ♀ above sterile ♂.


A
TOP
EXPERIMENT I
FEMALE —○
STERILE MALE —●
EXPERIMENT II
FEMALE —○
STERILE MALE —●
BOTTOM
B
TOP
EXPERIMENT I
STERILE FEMALE —○
MALE —●
EXPERIMENT II
STERILE FEMALE —○
MALE —●
BOTTOM
C
TOP
EXPERIMENT I
○—STERILE FEMALE
●— " MALE
EXPERIMENT II
○—STERILE FEMALE
●— " MALE
BOTTOM
0 10 20 30 40 50 60
0 1 2 3 4 5 6 7 8 10
DAYS
COLUMN LENGTH (cms)

irradiation.

These results tended to negate the possibility that a sterile male control would be successful on this species because of the drastic effect that irradiation had upon the male response to females. However, there was still the maintained response of fertile male to sterile female upon which a control system might be developed. It was at least encouraging enough to proceed another step and to demonstrate beyond doubt that a pheromone existed, was able to be collected in testable amounts and was useful in manipulation of beetle behaviour as indicated by bioassay.

4.1.3 Demonstration, collection, isolation and bioassay of pheromones of S. oryzae

4.1.31 Introduction

Sex pheromones appear to play an essential role in the mating behaviour of the stored-product Coleoptera (Burkholder, 1970). Section 4.1.2 indicated that a specific and possibly pheromonic communication system is operating between the sexes of the rice weevil. The uses and promises of pheromones in insect pest control have been discussed for more than two decades Shorey and Gaston, 1967; Jacobson, 1972; Wood et al., 1970; Beroza, 1970; Birch et al., 1974; Tette, 1974). Burkholder and Dicke (1966) reported the possibilities of using sex pheromones in detecting and controlling injurious stored product pests. Burkholder (1974) reviewed some of the practical applications of pheromones in stored products pest control.

Since Valentine (1931) first demonstrated the presence of sex pheromones in the yellow meal worm, Tenebrio molitor L., many workers have discovered sex pheromones of individual stored product insect species

and have reported their effects upon insect behaviour (Hope et al., 1967; Burkholder and Dicke, 1966; Yinon and Shulov, 1967; Finger (Bar Ilan) et al., 1965; Happ and Wheeler, 1969; Keys and Mills, 1968; Tschinkel et al., 1967; Brady and Smithwick, 1968; Traynier and Wright, 1972, 1973; Burkholder, 1970; Halstead, 1973; Menon and Nair, 1972; Kuwahara et al., 1975; Read and Haines, 1976; Read and Beevor, 1976; Greenblatt et al., 1976; Calvert and Corbet, 1976; Hammack et al., 1976; Ryan and O'Ceallachain, 1976).

This necessarily incomplete bibliography indicates the degree of interest in such natural compounds and the possibility of using them in manipulating pest populations. One brief reference (De, 1970 quoted by Jacobson, 1972) concerns S. oryzae and a collection of its pheromone.

Applied systems, using attractants of various kinds, have been variously successful, the notable successes being those with tephritid fruit flies, where the lure has mainly been based upon odours of food (Bateman et al., 1966). Any such lure system must be able to compete strongly with the stimuli in the natural habitat and this means that very extensive and deep knowledge of this natural situation must be available. Those natural systems where constraints occur that improve the chances of the competing applied lure are worth investigating as possibilities for integrated controls using attractants.

Competition of synthetic and natural attactants within a random complex is unlikely to be successful as a basis for applied controls. With these matters in mind, one might assess households and storages as places where useful constraints might exist already or be capable of rather easy development. The relatively restricted size of any one storage and the possibility of generating low pest populations as a starting point are two factors that are attractive in encouraging investigation of pheromone-based

control systems.

The investigation must begin with the demonstration of an effective pheromone, a task sequentially described in this section. The objectives were simply to demonstrate that a pheromone existed and to collect, isolate and bioassay it as a basis of assessing its role in the behavioural cycle of adult rice weevils. No tests of control systems using pheromone of S. oryzae were considered nor was identification of the compound or its synthesis deemed within the scope of the current studies. Nevertheless, an attitude to the possible use of a pheromone within a pest control system was developed as part of the original hypothesis, stated earlier.

4.1.32 Materials and Methods

Olfactometer: A two-way choice olfactometer (Fig. 4.7) was constructed so that air could be drawn through a filter across virgin insects or isolated pheromones and into a Y tube arena in which insects under test could be introduced.

Pheromone collection apparatus: A pheromone collection apparatus was modelled on that of Burkholder (Pers. Comm.) involving filtered air being drawn over virgin beetles and through a Porapak Q Column (Fig. 4.8).

The choice of the Porapak Q system of collecting volatile materials was due largely to the development of the method by Burkholder for work with stored products beetles. Rudinsky et al. (1973) also used this solid phase absorbent successfully to obtain small amounts of beetle pheromones and Kevin et al. (1975) also demonstrated that the technique was suitable for collecting air-borne organic compounds which served as models for insect pheromones.

Figure 4.7 A two-way choice olfactometer.

A = Tube connecting to suction pump.

B = Chamber for test insects.

C = Y- tube.

D_1 and D_2 = Chambers for bioassay material.

E_1 and E_2 = Air-flow meters.

F_1 and F_2 = Air intake valves.

X = Regulatory stop-cocks.

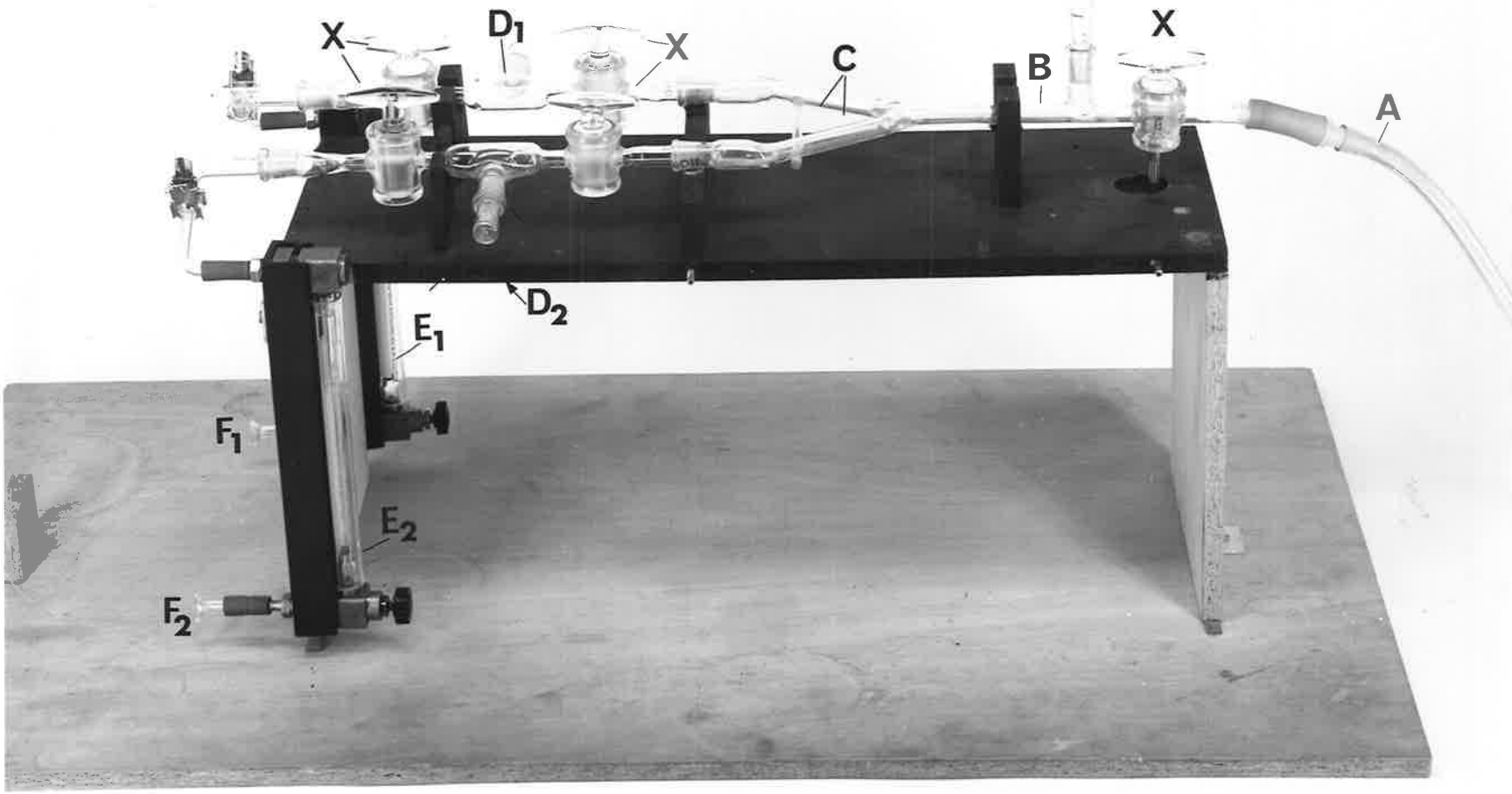
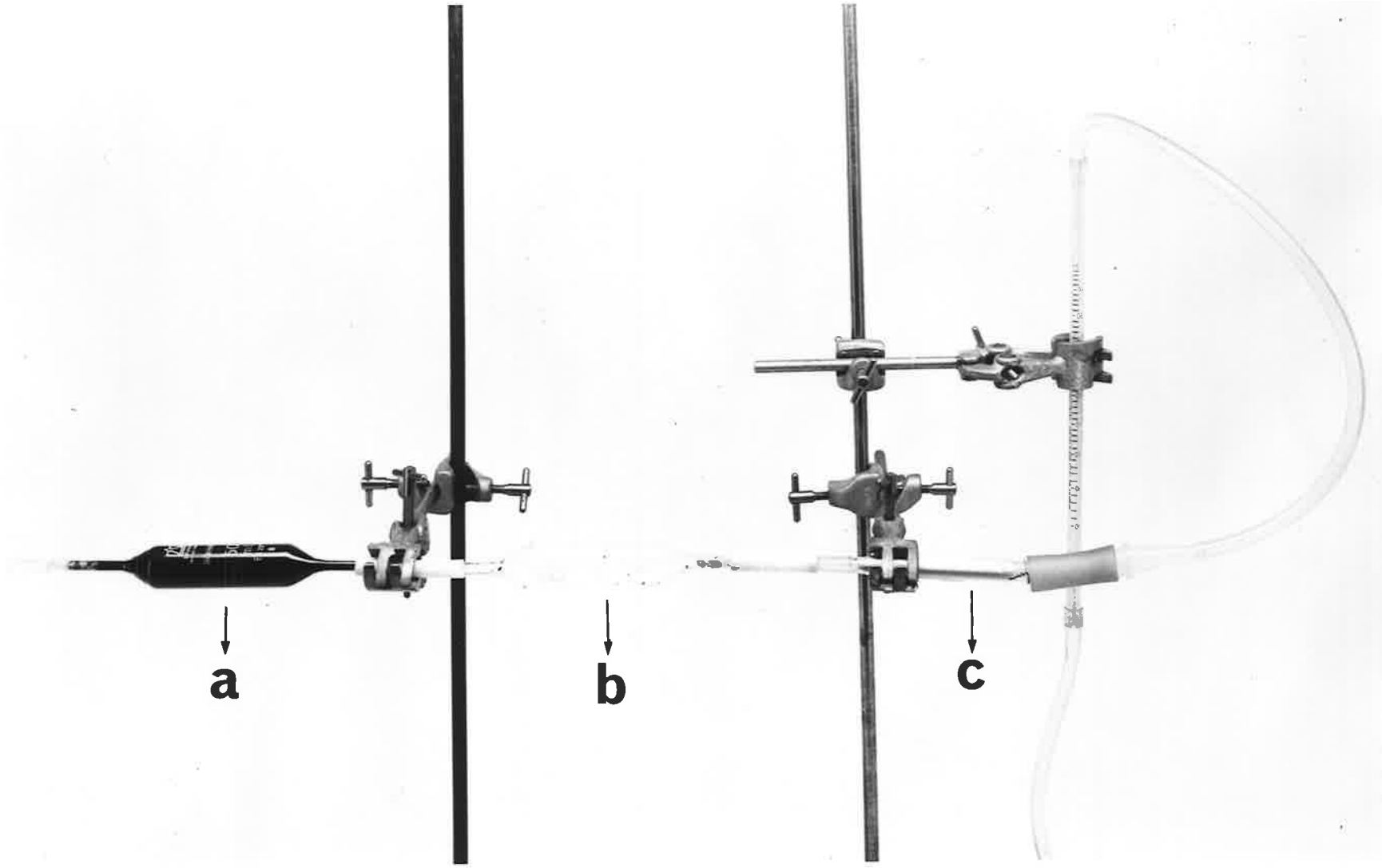

X
D1
X
C
B
X
A
D2
E1
F1
E2
F2

Figure 4.8 Pheromone collection apparatus.

a = Carbon filter

b = Insect chamber

c = Porapak Q column.


a
b
c

Porapak Q (ethylvinylbenze-divinylbenzene copolymer) 80-100 mesh was obtained from Waters Associates Incorporated, Farmingham, Mass., U.S.A.

The column was so constructed that it could be removed and reversed within the system allowing collected pheromone to be washed from the column with an appropriate solvent (Hexane). Once the column had been prepared (Appendix 4.1) and washed with the solvent it was not subjected to temperatures above 180^{o}C unless being cleaned in preparation for re-use.

Collection of Pheromone: Collections were made from 100 unmated adults of the same sex and age (4-5 day -old) group, over a period of 24 hours when a fresh group was introduced and the procedure repeated for one week.

Bioassay: Forty virgin males or females as responders were tested against 25 virgins of the opposite sex. They were selected for their age from emergence and beetles of the same age were tested against each other. Ages tested varied from <1 to >6 days providing 5 age-groups for the experimental series. Pheromone-producing virgins were placed with 5 gm of wheat in the bioassay chamber of the olfactometer. The control was 5 gm of wheat only.

In other studies 10 µl of extracted pheromone (solution of "pheromone" or material collected in the Porapak Q column + hexane) was used as the source and the control was 10 µl of hexane only. Ages of the responding insects tested were >1, 1-2, 3-4, 4-5 and 6-7 days.

Insects that had been sterilized by subjecting them to 10 Krads of ^{60}CO as described in Section 3 above were also tested against extracted material.

Results were subjected to the χ^2 test to determine the significance of response of the beetles.

4.1.33 Results and Discussion

Responses of virgin beetles to virgin beetles of the opposite sex:

The responses of beetles under olfactometric conditions indicated that for fertile insects:

(i) ♂ responded to ♀ (Table 4.3)

(ii) ♀ responded to ♂ (Table 4.4).

TABLE 4.3 Responses of 40 ♂ to 25 ♀ of similar ages in the olfactometer.

Age in days	Number of males responding	χ^2 Value	P
>1	21	0.10	N.S.
1-2	27	4.90	<0.05
3-4	28	6.40	<0.02
4-5	32	14.90	<0.001
6-7	29	8.1	<0.01

TABLE 4.4 Responses of 40 ♀ to 25 ♂ of similar ages in the olfactometer.

Age in days	Number of females responding	χ^2 Value	P
>1	24	1.60	N.S.
1-2	28	6.40	<0.02
3-4	29	8.10	<0.01
4-5	29	8.10	<0.01
6-7	28	6.40	<0.02

The insects of both sexes were not responsive when less than a day old. After this age the female tended to respond to males in about the same degree throughout the other age groups tested. Male response was greatest to females 4-5 day-old and this somewhat confirms the evidence of the experiments in columns of grain (Section 4.1.2).

Responses of virgin adults to material collected from females or from males in the Porapak Q Column (pheromone): Again males less than a day-old did not respond but older males responded better to pheromone extracted from 4-5 day-old females (Table 4.5). A similar result was obtained with the female response to pheromone extracted from males (Table 4.6).

TABLE 4.5 Responses of 40 males of varying ages to material collected in the Porapak Q Column (pheromone) from females.

Age in days	Number of males responding	χ^2 Value	P
>1	21	0.10	N.S.
1-2	27	4.90	<0.05
3-4	28	6.40	<0.02
4-5	30	10.00	<0.01
6-7	27	4.90	<0.05

Responses of sterile insects to material collected in the Porapak Q Column (pheromone): Both sexes of sterilized (irradiated) S. oryzae responded in much the same way as was demonstrated for fertile (non-irradiated) beetles (Table 4.7).

TABLE 4.6 Responses of 40 females of varying ages to material collected in the Porapak Q Column (pheromone) from males.

Age in days	Number of females responding	χ^2 Value	P
>1	22	0.40	N.S.
1-2	27	4.90	<0.05
3-4	27	4.90	<0.05
4-5	29	8.10	<0.01
6-7	29	8.10	<0.01

TABLE 4.7 Responses of both sterile and fertile adults of S. oryzae to material collected in the Porapak Q Column (pheromone) from their opposite sex (Forty 4-5 day-old insects per test).

Sexes (Sterile/Fertile)	Number of individuals responding	χ^2 Value	P
Males (Sterile)	28	6.40	<0.02
Females (sterile)	29	8.10	<0.01
Males (Fertile)	30	10.00	<0.01
Females (Fertile)	29	8.10	<0.01

The data provided a reversal of the situation noted in the irradiated male behaviour within columns of grain (Section 4.1.2).

The evidence from these studies emphasises the early indications of pheromonic communication. Moreover the response to volatile materials collected from virgin adults proves that a sex pheromone is involved and that each sex produces pheromone. Whether the pheromone in both sexes is the same substance or not is beyond the ability of the current data to determine. The presence of pheromone in S. oryzae's communication system does not rule out the possibility of the additional presence of auditory signals. These are known in other species that produce pheromone (Rudinsky et al., 1973; Rudinsky et al., 1976). That both sexes produce pheromone is hardly surprising as this is reported for Tenebrio molitor (Tschinkel et al., 1967), Trogoderma granarium (Finger (Bar Ilan) et al., 1965; Yinon and Shulov, 1967), and Tribolium confusum (Ryan and O'Ceallachain, 1976).

The effect of age on release of pheromone and responses to it is a common phenomenon in insects. The result on the effect of age on responses in the current study is well supported by those of Adeesan et al. (1969), Burkholder (1970), Calvert and Corbet (1973), and Kuwahara et al. (1975).

The clear positive responses of irradiated males of rice weevil to collected pheromone demonstrated that data from the columns of grain (Fig. 4.6 AI and II) incorrectly indicated impaired pheromonic responses. The positive responses of irradiated S. oryzae to pheromone are consistent with data available for other species (Bartlett et al., 1968; Menon and Nair, 1972; Stimmann et al., 1972). One interesting report of the variable effect of different methods of sterilizing insects is that on the cabbage looper where a chemosterilant used as a spray did not affect male response to females. The same material when fed to the looper reduced male response significantly (Henneberry et al., 1966).

The existance of effective pheromonic materials in S. oryzae is clearly demonstrated and their potential in aggregating both sexes of rice weevil has been shown to be correlated with the age of the adult. The materials can be collected and concentrated and are therefore good candidates for identification and perhaps synthesis. Synthetic compounds will need to be assessed against the natural compounds as collected in Porapak Q and as produced by virgin adults, before proper evaluation of their competitive success is possible. Nevertheless the basis for a pheromone type lure trap control system for S. oryzae at least in low density populations is demonstrated.

5. GENERAL DISCUSSION

The increasing instances of resistance of stored product pests to the insecticides currently used in their control (Parkin, 1965; Freeman, 1974) combined with cyclic harmful effects such chemicals may exert upon ecosystems (Carson, 1962) makes it essential to develop, where possible, safer and more ecologically acceptable alternatives in protecting the food resource upon which the world depends.

The hypothesis that generated these studies was derived from the above facts and concepts. It concerned the premise that insects infesting stored food might be controlled by a pest management programme that incorporated the sterile insect release method if the behaviour of the insects could be manipulated to offset the disadvantages of having to generate increased population density.

Because of its complexity, the components of the hypothesis were isolated and examined individually. Though these components may not be directly compared, they were linked into a logical sequence. The early biological studies enabled me to develop a relatively standard system of methodology for subsequent studies. Previous research on the rice weevil, S. oryzae and the lesser grain borer, R. dominica, particularly those of Birch (1945a, b, c, 1948, 1953) provided an independent check on my results. Studies such as, innate capacity for natural increase in population, is a case in point. Subsequent studies upon population development in the test insects were generally subjected to scrutiny in terms of their controls.

The radiation studies were straight forward and results were not unexpected when compared with the results reported in references consulted. Nevertheless one needed to know how the particular culture of insects available, reacted relative to increasing doses of irradiation,

and also how density and irradiation sterility, the two components of the system, affected populations.

Even though the results indicated the practicability of the sterile insect release method, its disadvantages (Crook et al., 1960; Erdman, 1974) as a control for stored products pests remain.

The radioisotope monitoring system for tracing grain beetles in columns of grain is based upon successful monitoring of dispersal of other species (Giles, 1969; Lamb et al., 1971). The results for rice weevil were indicative of the presence of pheromonic communication between the sexes. This stimulus may be used to manipulate the behaviour of rice weevil and may be useful in overcoming some of the disadvantages of the sterile insect technique in stored product pests control as suggested in Section 3. Disruption of communication between the sexes to prevent or reduce successful matings may also be possible (Burkholder, 1973). Such powerful attractants may also be used as lures or baits incorporated with insecticides, pathogens or sticky materials to capture and kill pests (Burkholder, 1974; Burkholder and Roush, 1974).

The apparent lack of response by irradiated males to females that occurred in grain columns (Section 4.1.2) was not confirmed in the olfactometric experiments. These opposing results require further investigations, particularly as the columns of grain represent a more natural medium in which behavioural stimuli must operate.

The individual discussions in the various sections presented highlight the results obtained and provide suggestions or tentative conclusions. In total the results may be brought together and summarized thus:

The test insects may be successfully sterilized by irradiation but the doses needed to ensure sterility of adults, cause a significant

reduction in longevity whilst not appearing to affect competitiveness with fertile or non-irradiated individuals. The density ratio (sterile to fertile males) required to inhibit development of new generations of a species is about 25:1 for S. oryzae and 35:1 for R. dominica.

Dispersal and pheromone communication, studied for S. oryzae only, demonstrated that this species could be manipulated when population density is very low. The total concept of integrating behavioural manipulation and sterile insect density ratios into a pest management programme for grain weevil control is therefore basically defined.

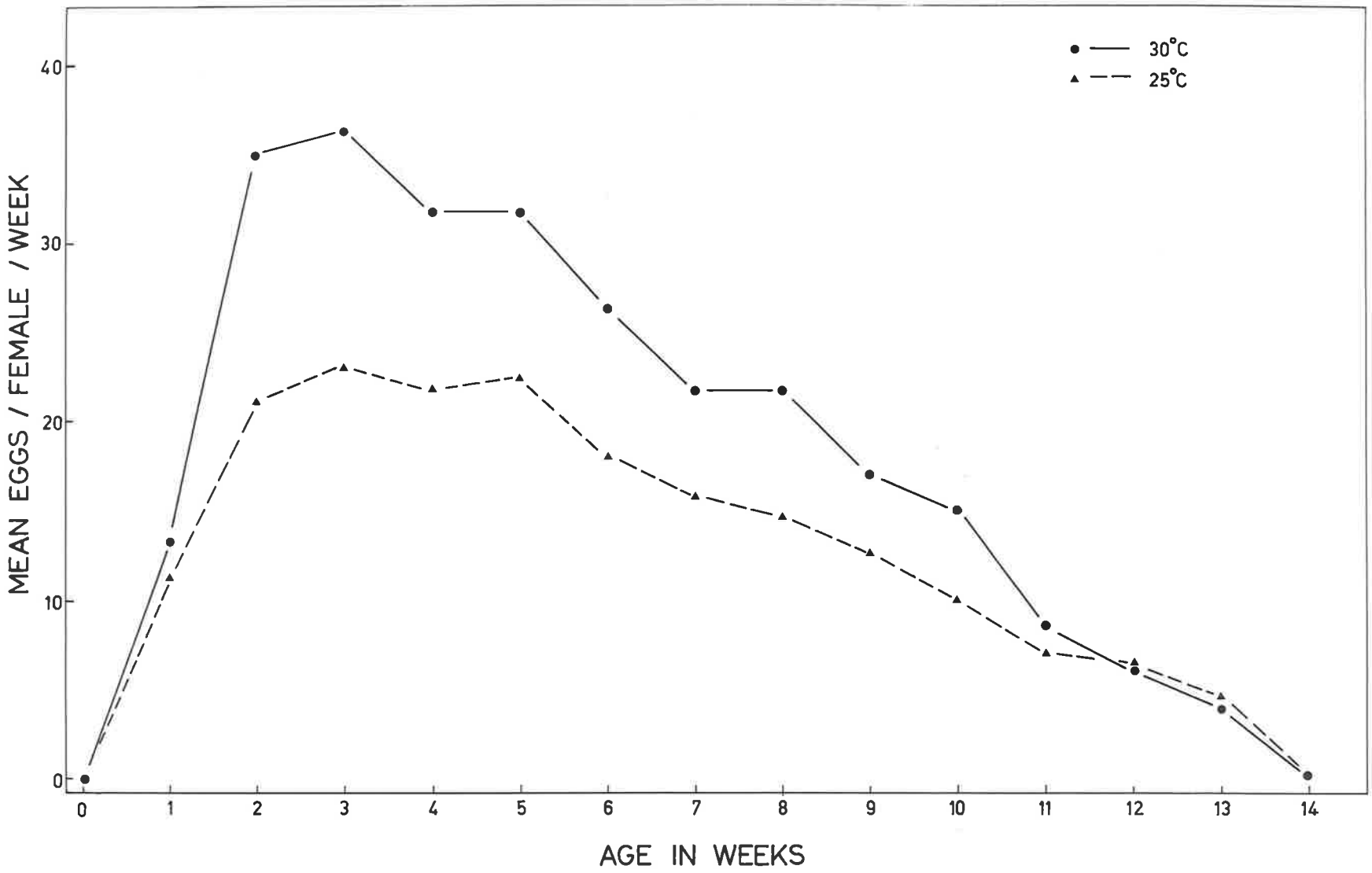
In practice it would be more realistic to think of combined control of grain pests such as is achieved with current chemical controls. The increasing trend of insect resistance to the chemicals available warns us to begin developing other control strategies with little delay.

Theoretically there are no reasons, why compounds of many pheromones should not be used in control processes; after all this is the natural situation in communities. The problems to be solved are basically concerned with learning the appropriate techniques of application. Part of this learning is to attempt constraints within natural systems that will improve the chances of the control applied - a synergism between technique and control processes.

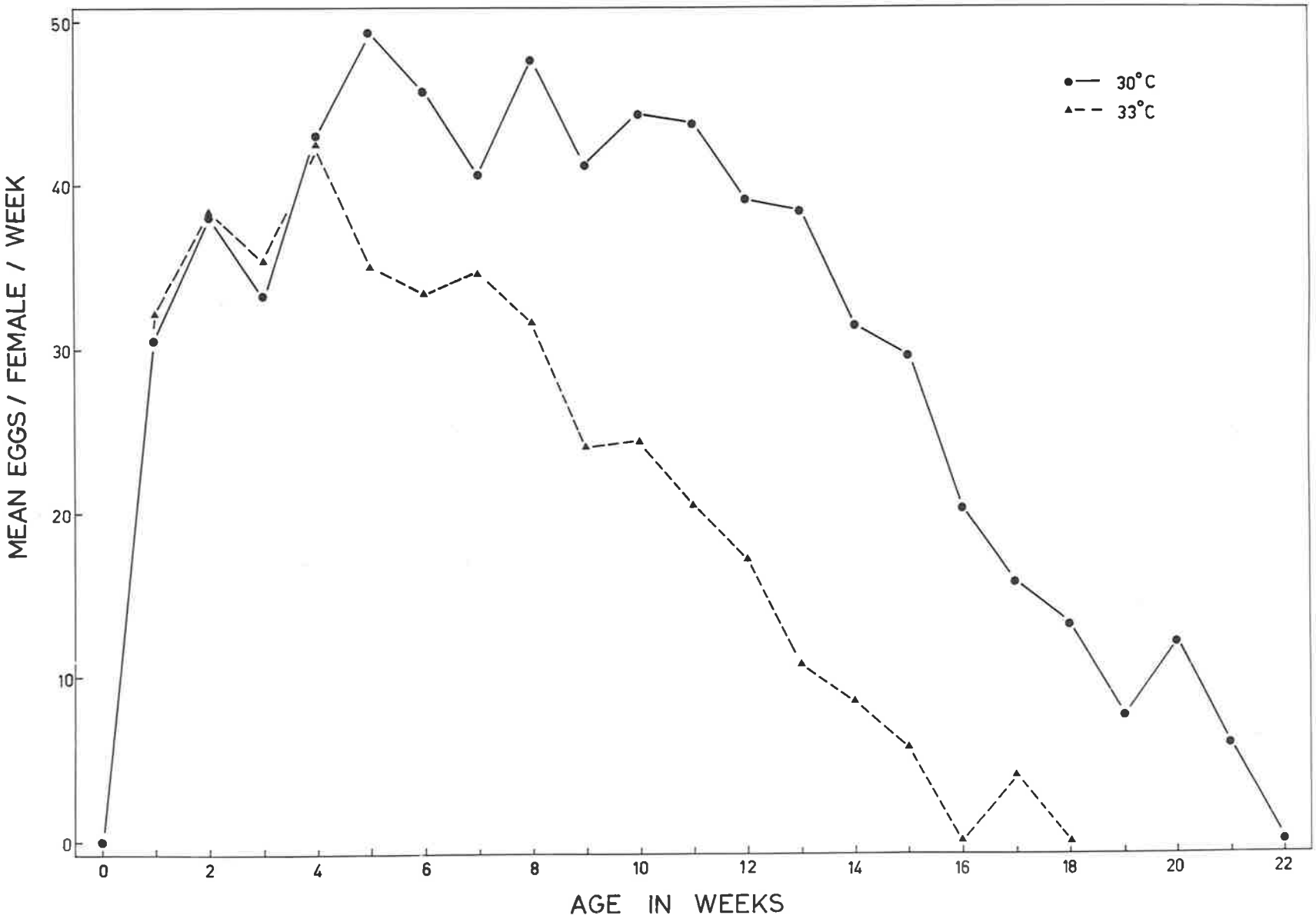
6. APPENDICES

For convenience the appendices have been numbered according to the sections to which they refer.

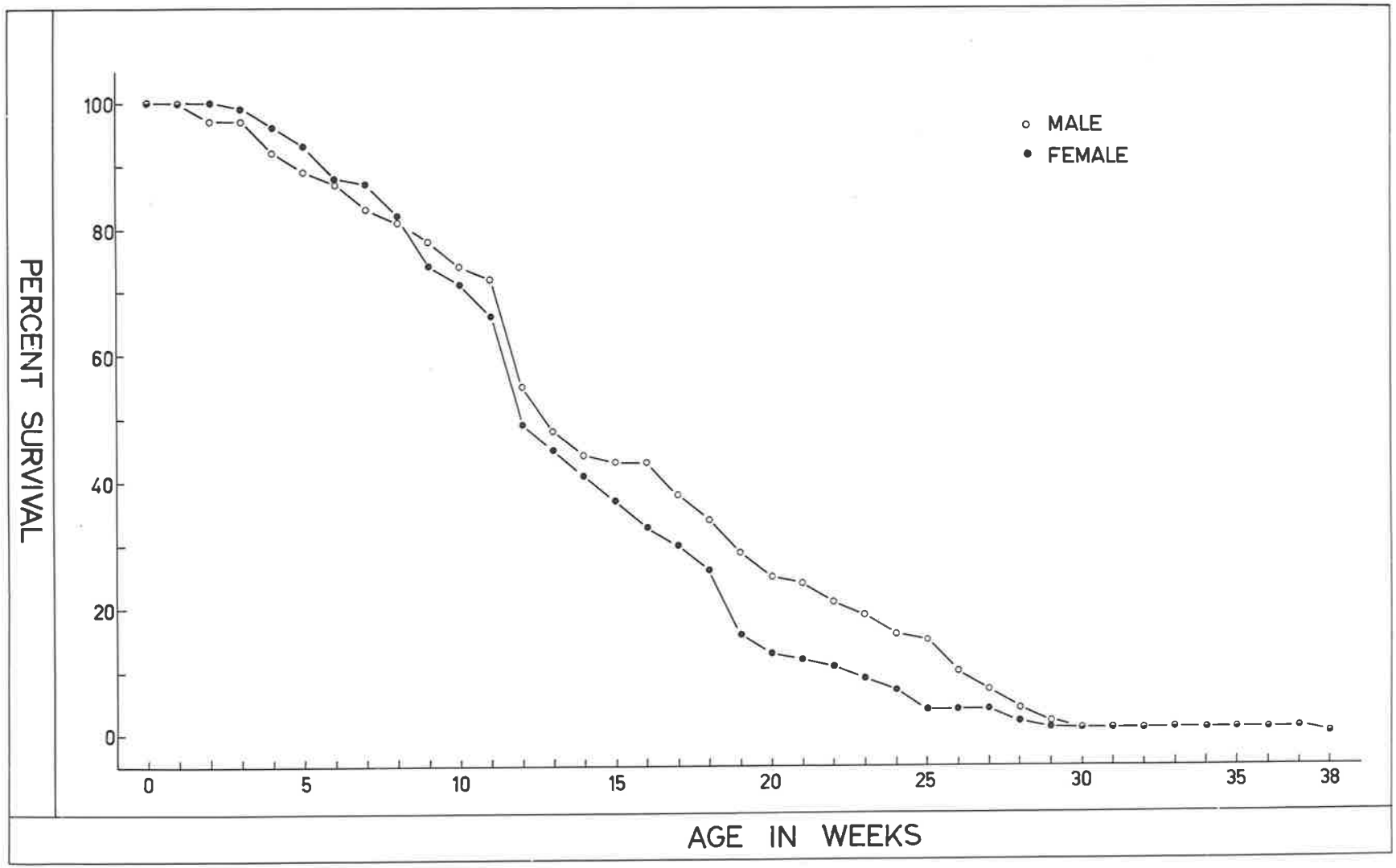
Appendix 2.1 The relative rates of oviposition by *S. oryzae* at 25°C and 30°C.


30°C
25°C
MEAN EGGS / FEMALE / WEEK
0
10
20
30
40
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
AGE IN WEEKS

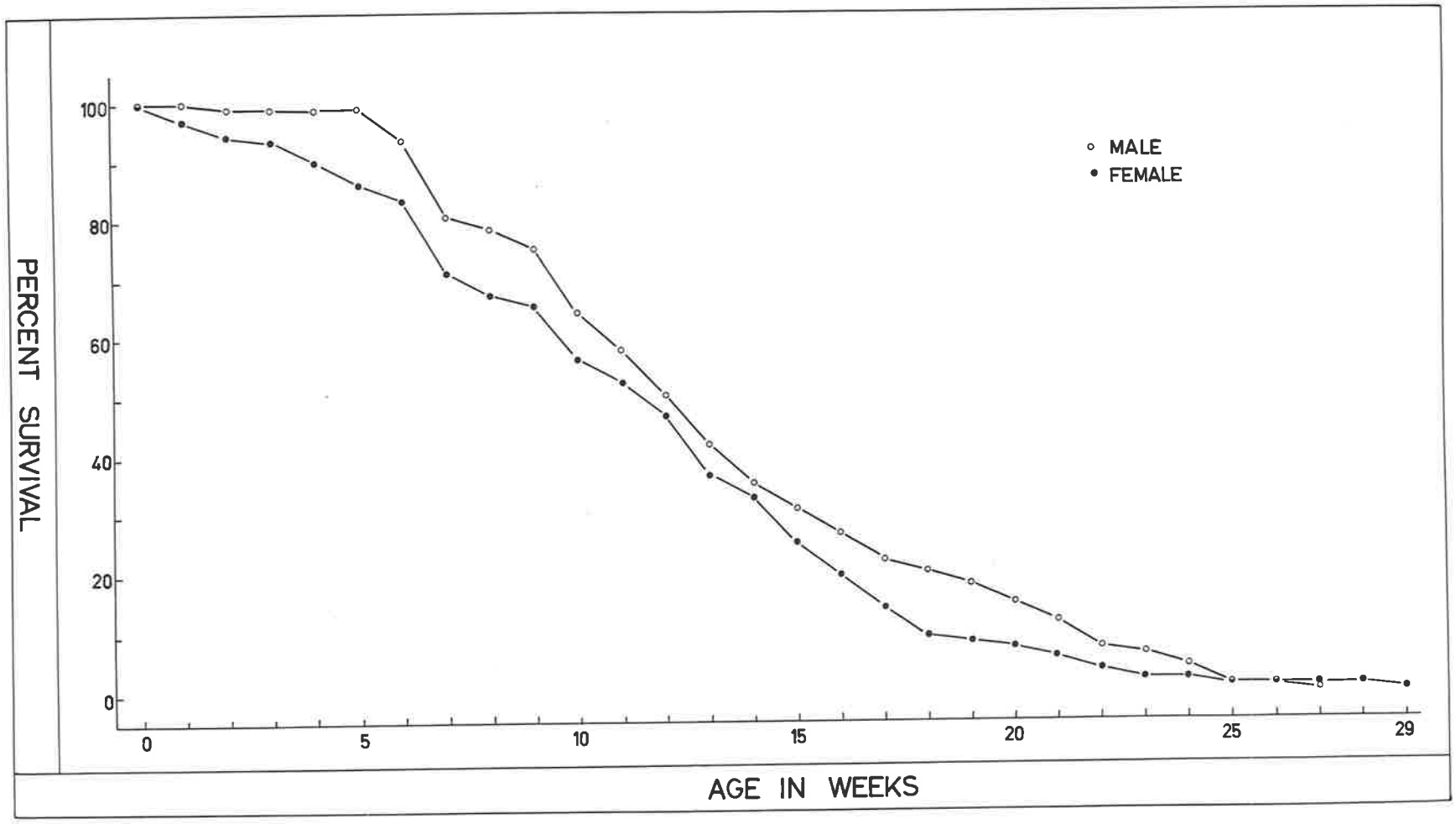
Appendix 2.2 The relative rates of oviposition by *R. dominica* at 30°C and 33°C.


MEAN EGGS / FEMALE / WEEK
AGE IN WEEKS
30°C
33°C
0
10
20
30
40
50
0
2
4
6
8
10
12
14
16
18
20
22

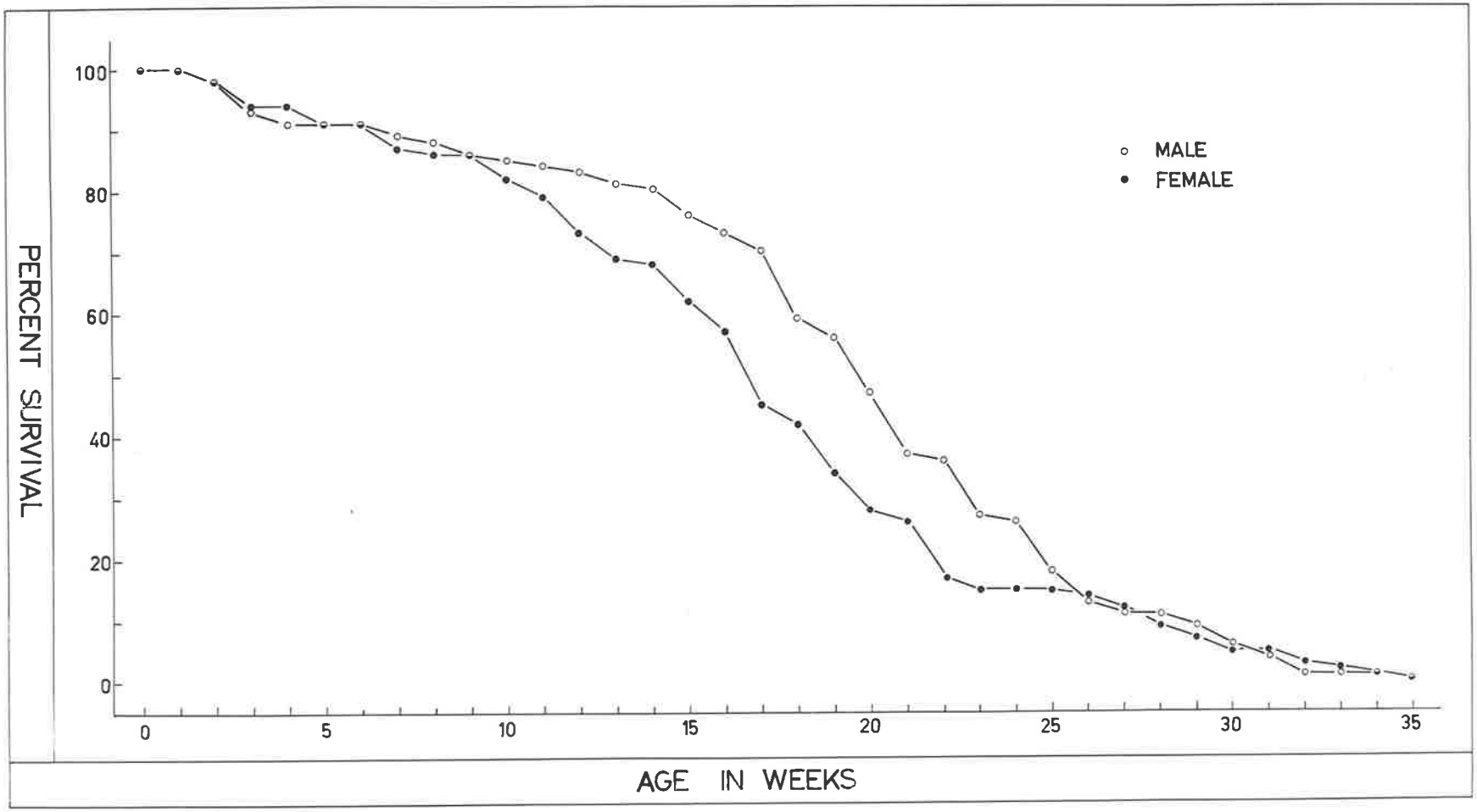
Appendix 2.3 Survival of adults of S. oryzae at 25°C.


PERCENT SURVIVAL
100
80
60
40
20
0
0
5
10
15
20
25
30
35
38
MALE
FEMALE
AGE IN WEEKS

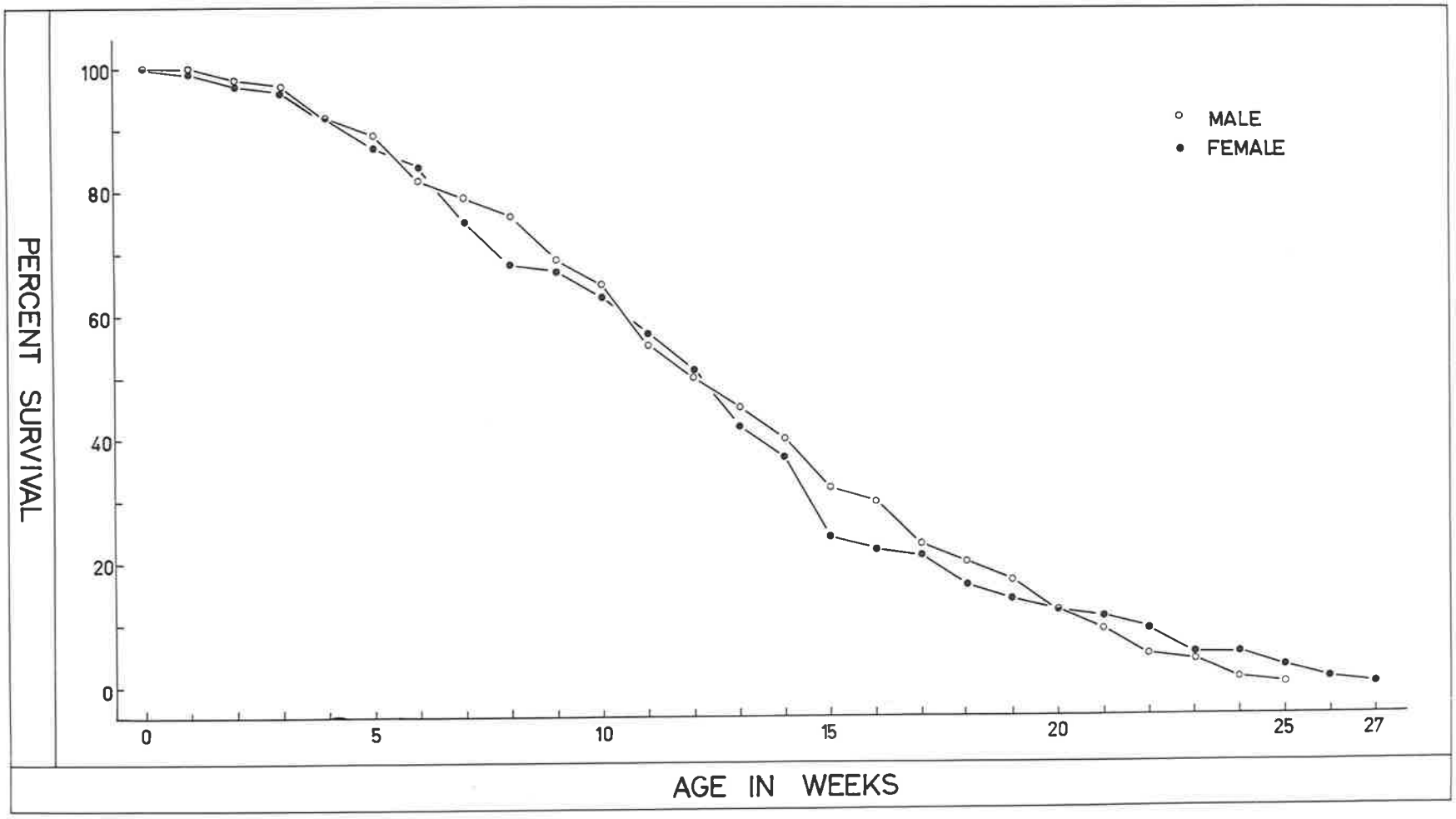
Appendix 2.4 Survival of adults of S. oryzae at 30°C.


PERCENT SURVIVAL
100
80
60
40
20
0
0
5
10
15
20
25
29
AGE IN WEEKS
MALE
FEMALE

Appendix 2.5 Survival of adults of R. dominica at 30°C.


PERCENT SURVIVAL
100
80
60
40
20
0
0
5
10
15
20
25
30
35
MALE
FEMALE
AGE IN WEEKS

Appendix 2.6 Survival of adults of R. dominica at 33°C.


PERCENT SURVIVAL
100
80
60
40
20
0
0
5
10
15
20
25
27
MALE
FEMALE
AGE IN WEEKS

Appendix 4.1 Preparation of Porapak Q column.

A Column of Porapak Q was prepared in an air condenser designed to fit into the pheromone collection apparatus. The porapak was held in place in the condenser by a glass wool plug at each end.

Prior to use the Porapak Q column was conditioned by heating it at 230^{o}C for 24 hours under a nitrogen flow of 200 ml min^{-1}. It was then washed with a solvent (hexane). The cleaned column was then heated again at 100^{o}C for 2 hours. It was then cooled at room temperature before use.

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