Pheromone Communication and Host-Finding Behaviour of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)

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Declaration

I certify that this work has not been accepted in substance for any degree, and is not concurrently submitted for any degree other than that of Doctor of Philosophy (PhD) the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise stated.

Tariq Bashir

to my loving parents

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Abstract

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) is a destructive pest of stored grain. Males produce a pheromone, with two components Dominicalure-1 (D1) and Dominicalure-2 (D2), which is attractive to both sexes. However, little is known about the pheromone biology of *R. dominica*. This thesis presents new studies that used behavioural bioassay and pheromone entrainment separately, and in tandem, to elucidate aspects of host finding behaviour, pheromone communication system and interactions between these two.

The role of host volatiles in primary host selection was tested for several different commodities. For the first time it was shown that *R. dominica* adults are unable to determine the suitability of a host from its volatiles alone. Further studies on the responses of beetles reared on two different hosts demonstrated that rearing medium does not affect beetle response to a host. The attractiveness of host grains, to both males and females, was increased when infested by male *R. dominica*. This affect was stronger for females. The mixture of host volatiles and aggregation pheromone was more attractive to both sexes than either of these alone.

Individual pheromone outputs of males varied considerably in the absolute quantities of pheromone components D1 and D2 but the ratio of the two in the blend varied little. Pheromone production was found to rise in the period 16.00h to 20.00h. The actual output of pheromone was positively correlated with body size and extent of feeding/boring.

When present with other males, *R. dominica* released smaller amounts of pheromone. However, when present in an unsuitable host or with females the pheromone signal was modified by a reduction in both the amount of pheromone released and proportion of D1 in the blend. Responding beetles found modified signals less attractive than 'normal' signals. Attempts were made to determine which characteristics of the signal were correlated with the observed responses.

The significance of these findings in relation to biology of *R. dominica,* possible practical implications and avenues for future research are discussed.

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Chapter: 1

General Introduction

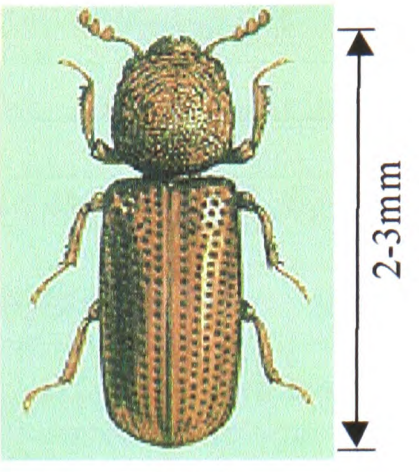
1.1. THE LESSER GRAIN BORER, *RHYZOPERTHA DOMINICA* (F.)

The lesser grain borer, *Rhyzopertha dominica* (Fabricius) belongs to the family Bostrichidae, super family Bostrichoidea, suborder Polyphaga, and order Coleoptera. The super family Bostrichoidea includes other families such as the Anobiidae, Dermestidae, Endecatomidae, Ptinidae etc. (Sikes, 1999; Bejsak-Collorado-Mansfeld, 2000). The family Bostrichidae includes another important insect pest of stored-grain, the larger grain borer, *Prostephanus truncatus* (Horn), which is very similar to *R. dominica* in appearance but larger in size.

1.1.1. Description & identification

R. dominica is a small sized grain-infesting beetle, just 2-3 mm in length. The adult (Figure 1.1a) is dark brown in colour with a tuberculate (knobly) prothorax. It has distinctive rows of punctures on the elytra. The antennae are ten-segmented and terminate in a large, three-segmented club.

Larvae (Figure 1.1b) are white in colour, 2.8 mm in length (full grown) and their C-shaped bodies are lightly covered with short setae (Potter, 1935). The head is almost completely hidden underneath the prothorax and only the powerful mandibles can be seen when viewed from above.



a. Adult

b. Larva

Figure 1.1: The lesser grain borer, *Rhyzopertha dominica* (Bostrichidae: Coleoptera)

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1.1.2. Importance of *R. dominica* as a pest of stored-grain

R. dominica is a destructive internal feeding pest of stored-grains (Figure 1.2) (Dowdy and McGaughey, 1992; Mayhew and Phillips, 1994). Both larvae and adults use their strong mandibles to attack whole, sound grains, causing extensive damage (Williams *et al.*, 1981). The adults chew grains voraciously and this not only causes weight loss (Brower and Tilton, 1973) but also reduces germination and vigour of the grains (Jilani *et al.*, 1989). It can also facilitate infestation by secondary pests and fungi (Mukherjee and Nandi, 1993). Grains seriously attacked by the beetle may be hollowed out until only a thin shell remains (Figure 1.2). More than one beetle may be found in one grain. The adults are strong fliers (Barrer *et al.*, 1993), and are therefore capable of infesting a grain store without being directly introduced from a contaminated source. It can feed on a variety of

food materials including grain of all kinds (Crombie, 1941), especially wheat, maize, rice and other cereals. Although it is considered a pest in stores, it has also been reported in the field (Sinclair and Haddrell, 1985). Being armed with powerful jaws the beetle can bore directly into wood that is regarded as its original food. It is also reported to eat its way into wooden and paper boxes and may destroy bookbindings (Potter, 1935). As the beetle is small and generally feeds inside the grain, it is difficult to detect the attack at the initial stage. After the attack has been established it is difficult to control as *R. dominica* is one of the most resistant of the stored-product insect species to pesticides. It has developed resistance to malathion (Champ, 1979), deltamethrin (Lorini and Galley, 1999), dichlorvos (Saxena *et al.*, 1999), phosphine (Mills, 1983; Sayaboc *et al.*, 1998; Alam *et al.*, 1999) and infrared treatments (Tilton and Schroeder, 1963). It has also shown intrinsic resistance against gamma radiation (Tilton *et al.*, 1966).

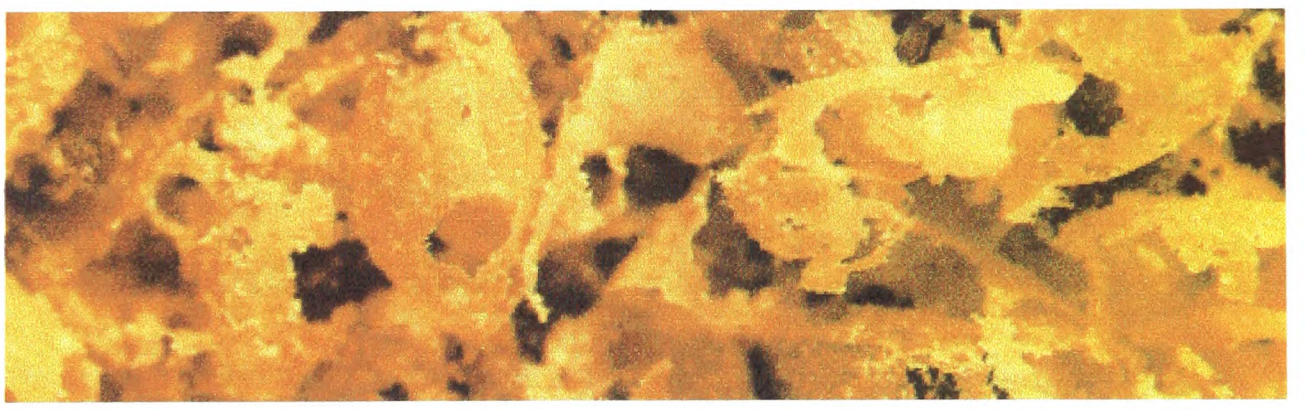


Figure 1.2. Photograph showing wheat grains seriously damaged by *R. dominica*; grains have been completely consumed except for the thin outer shell

1.1.3. Life cycle

The eggs which are 0.5-0.6 mm in length (Thomson, 1966) are laid either on the grain when they are generally laid in batches, or singly among the frass produced by the insects. Females may deposit up to 33 eggs per day (Thomson, 1966) and

200 to 500 eggs during their lifetime. The eggs are laid singly or in clusters of 2 to 30. The total developmental period from egg hatch to adult eclosion is on an average 58 days (Potter, 1935) but it varies considerably depending upon temperature and humidity, ranging from 29 to 81 days (Elek, 1994). The period before larvae hatch is about 7 days (Crombie, 1941). Eggshells of the hatched eggs mostly remain intact and can be easily detected. Cannibalism of un-hatched eggs by larvae can occur (Elek, 1994). First-instar larvae move over the grain and then chew their way into the kernel to reach endosperm, where subsequent development takes place (Osuji, 1982) or they may feed on the food particles left by other larvae and adults. Normally, larvae moult four times before pupation but occasionally the number of moults may vary from three to five (Potter, 1935) or even six to seven (Howe, 1950). Pupation usually occurs within the protective shell of the hollowed-out grain, but pupae may also be found in dust accumulation outside the inhabited foods. The normal pupation period is 10-12 days (Osuji, 1982). The pupa is white in colour with dorsal surface covered with hair. It exhibits the characteristics, depressed head and enlarged thorax of the adult (Barnes and Grove, 1916). The first mating normally take place at least 24 hours after the adult emergence. The adults are capable of living for at least several months and a few individuals may live up to fifteen months (personal observations).

1.1.4. Difference between sexes

The sex determination of *R. dominica* has always posed problems, as for a long time there were no recognisable external differences between adult males and females. According to Potter (1935) and Halstead (1963) the only suitable character for sexing occurs in the pupal stage. Potter (1935) in his comprehensive

paper on this beetle, reported that the genital papillae of pupae exhibit constant differences in each sex. At the end of the abdomen the male has a pair of two-segmented structures fused to the abdomen for their whole length while females posses two three-segmented papillae projecting from it.

Crombie (1941) proposed that adults of *R. dominica* can be sexed by squeezing the abdomen gently until the genitalia appear (Figure 2.2) which then may be examined under a microscope to determine the sex of the beetle. He stated that this technique had no adverse effect upon the insects. On the other hand, Birch (1945) felt that the technique was deleterious and should be chosen to examine the insects only at the end of an experiment. Sinclair (1981) showed that the squeezing method did indeed affect the insects, reducing both the longevity and fecundity. Stemley and Wilbur (1966) claimed that the colour characteristics of the fifth abdominal sternum of live adults could be used satisfactorily to sex beetles of this species. They stated that the last (5th) ventral abdominal segment of the female is pale yellow whereas the same segment of the male generally is uniformly brown. But Singh and Liles (1972), and Cline (1973) considered it an unreliable character. Ghorpade and Thyagarajan (1980) discovered a more reliable character for sexing the adult beetles. They reported the presence of a transverse shallow punctuate groove on the fifth abdominal sternum of the male, that is never present in the female (see section 2.3).

1.1.5. Distribution

R. dominica is thought to have originated from the Indian subcontinent (Potter, 1935) but now it is cosmopolitanly distributed (Aitken, 1975), as it has spread through commerce to all parts of the world. It is an important pest of stored-grain

in many countries with relatively warm climates (Cotton, 1956) such as Australia (Barrer *et al.*, 1993), Brazil (Lorini and Galley, 1996), Croatia (Kalinovic and Ivezic, 1994), India (Yadav, 1997), Malaysia (Rahim *et al.*, 1983), Nigeria (Osuji, 1982), Pakistan (Ahmed *et al.*, 1993), Taiwan (Peng and Peng, 1998) and USA (Fields and Phillips, 1994).

1.2. PHEROMONES

Pheromones are usually defined as olfactory messenger compounds, released by organisms to their environment, acting on target individuals of the same species (Karlson and Luscher, 1959). They include sex attractants, aggregation and alarm signals, trail or territory markers, oviposition deterrents or compounds that induce gamete release or control more complex social behaviour, and govern many other activities. Most of these are coded in complex multicomponent mixtures released in extremely small amounts. The potential of insect pheromones to be used as a component of integrated insect pest management strategies has given importance to the research in insect pheromones. Work done on different aspects of pheromones, such as their occurrence, isolation, determination of structure, synthesis, biological activity, effects on behaviour and use in plant protection has been reviewed by many authors (e.g. Bestmann and Vostrowsky, 1982; Birch and Haynes, 1982; Fadeev *et al.*, 1982; Leonhardt and Beroza, 1982; Burkholder and Ma, 1985; Tumlinson, 1988; Carde and Bell, 1995; Landolt and Phillips, 1997; Phillips, 1997; Vendilo and Lebedeva, 1998; Hardie and Minks, 1999).

1.2.1. Pheromones of storage insect pests

The first stored-product insect pheromone was identified nearly 33 years ago from the black carpet beetle, *Attagenus unicolor* (=*megatoma*) (Silverstein *et al.*, 1967). Since then many advances in our understanding of the pheromones of storage pests have been made (Burkholder, 1990). Now, pheromones are known from over 35 species of stored-product insects (Phillips, 1994; Plarre, 1998). As in other insects, pheromones of storage pests are generally volatile, low molecular weight organic compounds of various structures. Generally, pheromones of storage insect pests are classified as either sex pheromones or aggregation pheromones.

Sex pheromones

Sex pheromones are generally produced by one sex (usually the female) and attract members of the opposite sex for mating (Birch and Haynes, 1982). Among storage insects, female-produced sex pheromones are utilised by most of the moths, and by beetles in the families Anobiidae, Bruchidae and Dermestidae. The adults of these insects with sex pheromones generally tend to be relatively short-lived (days to weeks) and feed little (beetles) or not at all (moths) before they mate and die (Burkholder and Ma, 1985) but there are, however, exceptions.

Aggregation pheromones

Aggregation pheromones are generally produced by one sex (usually the male) and attract members of both sexes resulting in mating and aggregation at a food source (Phillips, 1997). Storage insects with male-produced aggregation pheromones may be found in the families Bostrichidae, Cucujidae, Curculionidae and Tenebrionidae

and these insects feed substantially and are relatively long-lived as adults (weeks to months) (Burkholder and Ma, 1985).

1.2.2. Use of pheromones in insect pest management

Pheromones are now an established tool for insect pest management although the extent of their use is still small compared with that of conventional insecticides. The most common use of pheromones is as attractant lures in traps to detect the presence of pests and to monitor the activities of the pest populations (Cogburn *et al.* 1982; Galbreath *et al.*, 1982a; Galbreath *et al.*, 1982b; Sinclair and Howitt, 1984; Phillips, 1997). Galbreath and Dale (1982) reported the use of insect pheromones for plant quarantine purposes.

Various studies have demonstrated the use of pheromones to control insect pests of different field crops such as cotton (Campion, 1994; Nassef *et al.*, 1999), sugarcane (David *et el.*, 1985), rice (Cork and Basu, 1996; Cork *et al.*, 1998; Su *et al.*, 1999) and maize (Hall *et al.*, 1981). Pheromones have also been used to manage insect pests of forest trees (Shea, 1995). The major groups of insects presently being controlled with pheromones are Lepidoptera (Campion, 1980, Khidr *et al.*, 1985) and Coleoptera (Burkholder, 1970). The main methods being used are mass trapping (Beevor *et al.*, 1993; Mafra and Habib, 1996; Reddy and Urs, 1997; Pfister, 1999) and mating disruption (Russell and Radwan, 1993; Carde and Minks, 1995; Fadamiro and Baker, 1999; Kehat *et al.*, 1999; Thorpe *et al.*, 1999). Combinations of pheromones with conventional insecticides have also been tested as a potential method of insect pest control. This technique called "attracticide" (attract and kill) has gained support from experimental studies in the recent past (Downham *et al.*, 1995; Brockerhoff and Suckling, 1999; Trematerra *et al.*, 1999).

Attempts to control insect pests of stored products have met with mixed success (Sinclair and Howitt, 1984; Campion *et al.*, 1987; Buchelos and Levinson, 1993; Suss *et al.*, 1999). However, pheromone traps provide an easy, efficient and extremely sensitive way to detect insects in storage facilities (Buchelos and Papadopoulou, 1999) and managers can use information from traps to locate infestations and make management decisions. The use of pheromone traps for monitoring populations needs careful considerations e.g. trap design and trap position etc. (Rejesus and Butuason, 1988; Smit *et al.*, 1997; Mullen *et al.*, 1998). The main use of pheromones for insect pest management in stores remains as attractant lures on traps to:

- detect the presence of insect pests,
- monitor the activities of pest populations,
- optimise the timing of other pest control operations, and
- check the efficiency of these control methods.

1.3. PRINCIPAL OBJECTIVE OF THE STUDY

The principal objective of this study was to investigate the pheromone communication and host-finding behaviour of *R. dominica* and interactions between these to gain insights into the function of these systems.

1.4. BACKGROUND TO THE STUDY

1.4.1. Management of *R. dominica*

Insecticides have been the main tool in combating pests for the last 50 years. The benefits these pesticides have brought to mankind are remarkable in terms of

increased net food production. However, through the widespread and sometimes indiscriminate use of pesticides, a number of problems have arisen such as premature resistance of insects to insecticides, outbreaks of secondary pests, pest resurgence, health hazards, environmental pollution and disruption of ecological systems. Realising the limitations of insecticides, the emphasis has now been shifted to plant resistance to insects, novel biochemical targets, and new approaches for pest control (Casida and Quistad, 1998). *R. dominica,* which is a very important pest of stored-grains and has shown resistance to many pesticides (see section 1.1.2), needs urgent attention in this regard. Improved pest management strategies with a priority to environmental protection and human safety need to be developed against this pest.

Various methods other than conventional pesticides that have been applied to control this pest include:

- combination of gamma and infra-red radiation or gamma and microwave radiation (Kirkpatrick *et al.*, 1973),
- plant oils (Jilani and Saxena, 1990; Shaaya *et al.*, 1991; Mohiuddin *et al.*, 1993),
- neem (*Azadirachta indica* A. Jussieu)-based insecticides (Jilani and Saxena, 1990; Rahim, 1998; Muda and Cribb, 1999; Sharma, 1999),
- exposure to the juvenile hormone analogue, methoprene, to reduce fecundity (Daglish and Pulvirenti, 1998),

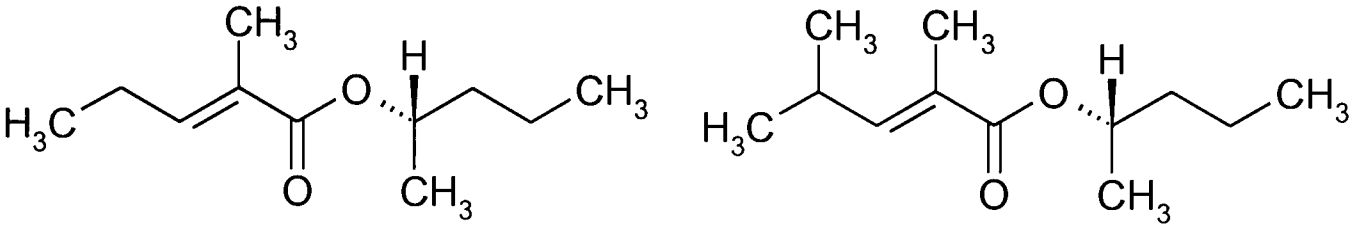
- parasitoids such as *Choetospila elegans* (Westwood) (Flinn, 1998), *Anisopteromalus calandrae* How. (Ahmed, 1996), *Cephalonomia waterstoni* (Gahan), and *Choetospila elegans* (Westwood) (Flinn *et al.*, 1996),
- pathogens such as bacteria, *Bacillus thuringiensis* (Beegle, 1996) and fungi, *Beauveria bassiana* (Balsamo) (Moino *et al.*, 1998; Rice and Cogburn, 1999),
- freeze-dried concentrated form of *Pseudomonas syringae*, an ice-nucleating active bacteria to decrease the cold-hardness of insects (Lee *et al.*, 1992), and
- chitin synthesis inhibitor, an insect growth regulator, to increase mortality of the immature stages (Elek, 1998).

Maximum impact of these and any other control methods can not be achieved, however, without complete knowledge of the biology and behavioural ecology of this pest. The pheromone communication system and host-finding behaviour of *R. dominica* are important parts of its biology and play a vital role in its survival and establishment. Knowledge of the function of these systems still remains fragmentary but could provide the basis of highly selective techniques for its control.

1.4.2. Aggregation pheromone of *R. dominica*

The male-produced aggregation pheromone of *R. dominica* was first reported by Khorramshahi and Burkholder (1981) and later isolated and identified by Williams *et al.* (1981). The pheromone was found to be made up of two unsaturated esters, (*S*)-(+)-1-methylbutyl (*E*)-2-methyl-2-pentenoate and (*S*)-(+)-1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate (Figure 1.3), which were given the trivial names of Dominicalure-1 and Dominicalure-2, respectively. The ratio of the two pheromone

components of *R. dominica* is highly variable between the pheromone entrainments (Mayhew and Phillips, 1994). There is also a considerable variation in the actual quantities of the two components released by males.



Dominicalure-1 Dominicalure-2

Figure 1.3. Chemical structure of pheromone components of male-produced aggregation pheromone of *R. dominica* (Williams *et al.*, 1981)

The aggregation pheromone of *R. dominica* has been synthesised (Cheskis *et al.*, 1985; Liu and Lin, 1990; Razkin *et al.*, 1996) and is being used as lure in pheromone traps. Fields *et al.* (1993) and Fields and Phillips (1994) used pheromone traps baited with to study the distribution of *R. dominica* in Canada. Krall (1984) used pheromone traps baited with "Trunc-call", the aggregation pheromone of *P. truncatus*, a species taxonomically related to *R. dominica*, to monitor its dispersal in West Africa. Hodges *et al.* (1983) used components of aggregation pheromone of *R. dominica* Dominicalure-1 and Dominicalure-2 individually and as a 1:2 mixture to monitor *R. dominica* and *P. truncatus* in farm maize stores in Tanzania. Mills and White (1994) used pheromone flight traps to study seasonal occurrence of *R. dominica* outside and within a southern Manitoba feed mill. Leos-Martinez *et al.* (1987) used probe traps baited with 50 µl of Dominicalure-1 released from rubber bands in bagged grains, to attract *R. dominica* and reported Dominicalure was a powerful attractant both inside a warehouse and outdoors for aerial trapping. Dominicalure-1, Dominicalure-2 and their mixtures

were equally attractive. On average, pheromone traps captured 8- and 152-fold more insects than unbaited control traps inside the warehouse and outdoors, respectively. Sinclair and Howitt, (1984), Rejesus and Butuanon (1988) and Fargo *et al.* (1994) tested the efficacy of pheromone traps for *R. dominica* and some other stored-product insects against traps baited with food-grain. They reported that insect catches were higher in pheromone traps than the grain traps in all the cases. Trematerra and Daolio (1990) studied the role of synthetic Dominicalure to attract non-target species, and the effect of trap position on its efficiency. They reported that *Cryptolestes ferrugineus* (Stephens), *Sitophilus oryzae* L. and *Colydium casteneum* (Herbst) were also caught on the traps baited with pheromone of *R. dominica*. However, the number of these insects caught on traps varied considerably with the position of the trap. Considerably more insects were trapped on the traps placed at brighter places than those placed at dim places.

1.4.3. Pheromone biology of *R. dominica*

Pheromone production over time by male *R. dominica* and the effects of feeding, food nutritional value, mating and population density on its production were investigated by Mayhew and Phillips (1994). The pheromone was collected through aeration using the solid-phase adsorbent, Super-Q. Volatiles were collected for 24-hour periods and quantified using gas chromatography. Pheromone was produced 3-5 days after feeding and once started, production did not cease over the course of one month. The onset of pheromone production following feeding was on average 4.71 days $\pm$ 1.06 (SE). In a 24-hour entrainment the maximum amount of Dominicalure-1 was 1114.756 ng $\pm$ 109.9 (SE), occurring 18 days after feeding and maximum amount of Dominicalure-2 was 960.377 ng $\pm$

78.0 (SE), occurring 14 days after feeding began. They stated, without giving any data, that pheromone production increased proportionally as the content of wheat flour increased relative to non-nutritive cellulose in the food substrate. Clearly, this observation needs to be confirmed. *R. dominica* males produce pheromone signals after arriving at a food source and conspecific individuals respond to those signals and aggregate at the food source. In this case, one would expect them to produce pheromone signals if other individuals are need to be attracted at the food source and not if they are not, i.e. an all or nothing signal. Therefore, production of a smaller quantity of pheromone on a low quality food is surprising because if the other individuals are not need to be attracted due to unsuitability of the food the pheromone signals should not be produced at all.

Mayhew and Phillips (1994) demonstrated that pheromone production in *R. dominica* is dependent on feeding. However, it is not known whether feeding simply triggers pheromone production or rate of pheromone production is associated with rate of feeding, and whether pheromone signals contain any information about the suitability of the host. In their study, pheromone production levels between mated and unmated males of the same age were not significantly different. However, they suggested that, as the mating system of *R. dominica* is not fully understood, it is not certain that the effect of mating on pheromone production was adequately tested as their experimental method involved one *R. dominica* male paired with only one female.

Studies using synthetic pheromone has shown that, *R. dominica* adults are more strongly attracted to components, Dominicalure-1 and Dominicalure-2 of the aggregation pheromone than to their optical isomers (Selitskaya and Shamshev,

1994), and individual components and various mixtures are equally attractive (Cogburn *et al.*, 1984). In contrast, it has been reported for the related species, *Prostephanus truncatus*, that the major attractant is Trunc-call 2 and that by itself Trunc-call 1 attracts few beetles (Leos-Martinez *et al.*, 1995) but in situations where the concentration of Trunc-call 2 appears to be high then a mixture with Trunc-call 1 is significantly more attractive (Hodges et al., 1998). Very few studies have been made to test the response *R. dominica* adults using natural sources of pheromone (Dowdy *et al.*, 1993), and there seems to be no studies using single males as pheromone source.

It is evident from this brief review that, in most of the studies that have been carried out so far on the pheromone of *R. dominica*, the main emphasis was on the practical use of pheromone for monitoring and control of the beetle. Only a few efforts have been made to study the more basic aspects of the relationship between *R. dominica* and its aggregation pheromone. For example, what are the different factors that can affect the production of pheromone signals by males, and what are the factors that can affect the response of conspecific males and females towards these signals? What is the biological function of the pheromone, to attract females (Otte, 1974) or both males and females (Borden, 1982)? Has it evolved in the context of mating behaviour (Raffa *et al.*, 1993)?

1.4.4. Host-finding behaviour of *R. dominica*

Little is known about the host-finding behaviour of *R. dominica*. It has been reported that it is attracted to wheat volatiles in laboratory experiments (Crombie, 1941; Dowdy *et al.*, 1993) but there are many questions still to be answered. Can it select a suitable host on the basis of host volatiles? Can it discriminate between

volatiles of a suitable and an unsuitable host? Only males have the ability to communicate through the pheromone signals to conspecifics about the availability of a food source, are they also more efficient than females at locating a food source? Are the pheromone signals produced on different types of hosts similar?

1.5. OBJECTIVES OF THE STUDY

The two main approaches used in this study to investigate the pheromone communication system and host-finding behaviour of *R. dominica* were analysis of quantity and composition of pheromone produced by males and determination of behavioural responses of males and females to different odour sources. Questions concerning how an animal behaves and why it does so may not have obvious answers. The first step towards understanding animal behaviour involves posing appropriate questions (Foster and Harris, 1997). Keeping in view the points discussed above the main questions addressed in this study were:

- Can adults of *R. dominica* select a suitable host on the basis of host volatiles alone?
- Is response to host volatiles affected by the presence of aggregation pheromone?
- Do pheromone signals contain information about the quality/suitability of the host?
- Do individual male signallers vary in their pheromone signals?
- Does feeding simply trigger pheromone production, or is the rate of pheromone release associated with rate of feeding/boring activity?

- Is pheromone production by males affected by the host-type?
- Is pheromone production by males affected by the presence of the conspecifics?
- Is there any difference between the behavioural responses of beetles to the male signallers present in different types of host-grains?
- Is there any difference between the behavioural responses of beetles to the male signallers present alone or with females?
- Is there any difference in the behavioural response to pheromone signal between males and females?
- What characteristics of a pheromone signal affect the response of the beetles?

1.6. THESIS PLAN

Chapter 2 gives the general materials and methods relevant to all the experiments, which are reported in Chapters 3 to 8.

Chapter 3 reports the results of the olfactometer tests undertaken to observe the behavioural response of walking *R. dominica* adults towards different host volatiles (i.e. wheat, maize, groundnut, de-oiled groundnut and groundnut oil) to determine whether this insect could distinguish the volatiles of a suitable host from a less/unsuitable host. This chapter also reports the results of the olfactometer tests undertaken to investigate the effect of past experience on the behaviour of *R. dominica* in relation to host selection/preference using wheat and split green gram as two contrasting hosts.

Chapter 4 reports the results of the experiments that addressed the questions, is the response of *R. dominica* adults to host volatiles modified in the presence of

aggregation pheromone, and is the combination of host volatiles and pheromone more attractive than pheromone alone (male released rather than synthetic).

Chapter 5 investigates the variation among individuals in the absolute quantities and ratio of the pheromone components produced by *R. dominica* males and the relationship between the rate of feeding/boring activity and the rate of pheromone production. This is done by quantifying pheromone output from single males.

Chapter 6 reports studies investigating the effect of host type on production of pheromone signals by *R. dominica* males, and measures the responses of males and females to these pheromone signals produced by males present in a suitable host (maize) or in an unsuitable host (groundnut).

Chapter 7 reports studies investigating the effect of presence of conspecifics on production of pheromone signals by *R. dominica* males, and measures the responses of males and females to the pheromone signals produced by lone males and males present with females.

Chapter 8 reports the results of the experiments undertaken to determine which characteristic(s) of a pheromone signal is most correlated with the observed response of male and female *R. dominica* adults, using synthetic pheromone.

Chapter 9 is a general discussion of results obtained during these studies. It attempts to draw the experimental results together to obtain a better understanding of the pheromone communication system and host-finding behaviour of *R. dominica*. It also discusses the biological function and evolutionary significance of the male-produced aggregation pheromone in this insect, the potential practical implications of these findings and potential future research.

Chapter: 2

General materials and methods

2.1. GRAIN COMMODITIES

Wheat and wheat flour [*Triticum vulgare* L.]

Whole organic English wheat and wheat flour were supplied by Canterbury Wholefoods, 10 The Borough, Canterbury, Kent, UK.

Maize [*Zea mays* L.]

Whole organic yellow maize was supplied by Gillet and Cook Ltd., Monks Granary, Standard Quay, Faversham, Kent, UK.

Groundnut [*Arachis hypogaea* L.]

Groundnut kernels (produce of China) were purchased from Holland and Barrett, Chatham, Kent, UK.

Green gram [*Vigna radiata* (L.)]

Organic green gram (split) were supplied by Canterbury Wholefoods, 10 The Borough, Canterbury, Kent, UK.

2.2. THE INSECT

A strain of the lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) from Pakistan was used in these studies. The strain was originally collected from Pakistan in October 1994 and subsequently reared at NRI.

2.2.1. Insect rearing

The stock cultures of *R. dominica* were reared on organic English wheat grain in a CTH room set at 27±1°C, 60±5% r.h. and 12 hour light/dark cycle. Wheat grain was frozen, stored in a cold room (3-4°C) and then equilibrated to room temperature of 20±5°C before being used for culturing insects. Cultures were started by introducing approximately 200-300 unsexed adults into a 2.5 litre jar containing approximately 1.5 kg of wheat. The jars were closed with black 'Rund' filter paper (Schleicher and Schuell, Germany) sealed at the edges using molten wax. Black filter paper was used for easy observation of possible mite or psocid infestation. To prevent infestation by mites and psocids, each jar was placed on an up-turned saucer placed on a tray containing paraffin oil. Fresh cultures were started every 9-10 weeks. Under these conditions, development from egg to adult took approximately 40 days.

Special sub-cultures were prepared to obtain unmated males and females. About 100 unsexed beetles were introduced into a 2.5 litre jar containing a 1 kg mixture of whole wheat flour and brewer's yeast (10:1) and kept in the CTH room as stated above. Parent adults were removed from the culture after seven days, and thirty days after their removal the cultures were sieved daily through 710 μm sieves (Philip Harris Scientific, London) to remove all newly emerged adults.

2.2.2. Preparation of insects for experiments

The adults that were removed from the culture within twenty-four hours of emergence were assumed to be virgin (Dowdy *et al.*, 1993; Mayhew and Phillips, 1994). After removing from the culture, beetles were kept singly on kibbled wheat grains until used in the experiments. Kibbled wheat grains were used to allow easy recovery of the insects. Seven-day old beetles were used in all the experiments, unless stated otherwise.

The beetles whose response to volatiles was observed in the olfactometer experiments were generally allowed to feed on kibbled wheat grain for six days, then starved for twenty-four hours, unless stated otherwise, before being used in the experiments.

The male beetles used as potential signallers in pheromone entrainments, were generally allowed to feed on kibbled wheat grains for three days. Then they were moved into the host-grains (already prepared by drilling holes in them with 1.5 mm drill) and were allowed to feed there for four days, unless stated otherwise, before using them in the experiments.

2.3. SEX DETERMINATION

Adults were generally sexed by examination of the tip of the abdomen using a binocular microscope. Adult males were recognised by the presence of a punctuate groove on the fifth abdominal sternite (Ghorpade and Thyagarajan, 1980) (Figure 2.1). This groove, generally present on both sides or at least on one side of the mid-ventral line, is rather shallow and of variable development. This sort of

groove is never present in the females in which the sternum is more convex than in the male.

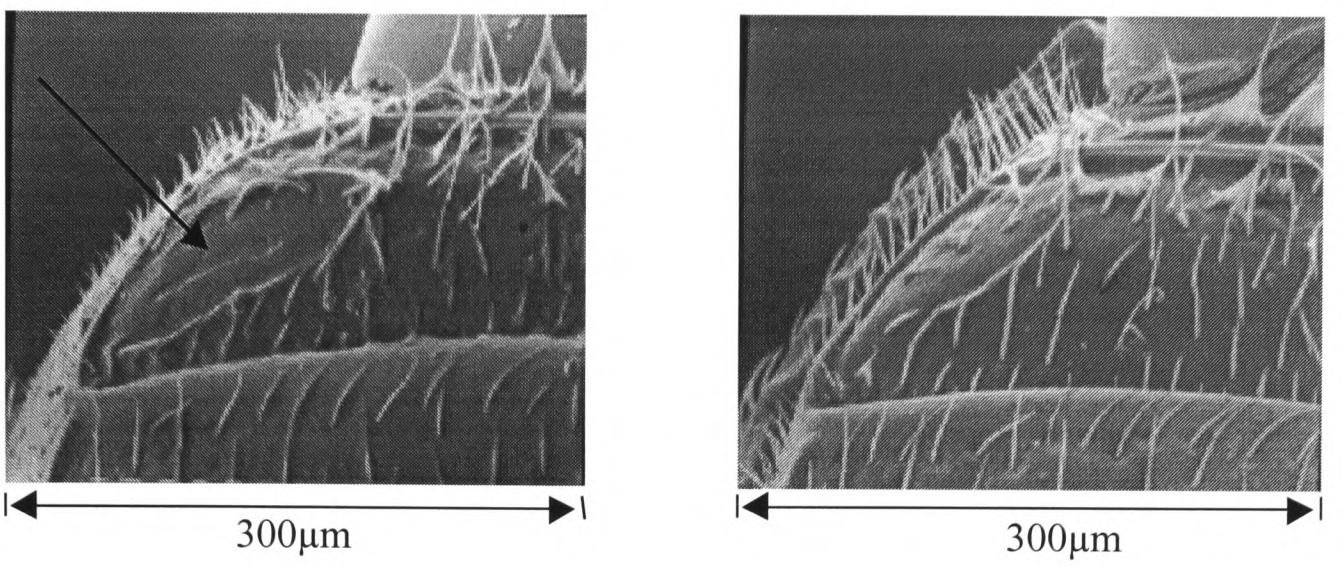


Figure 2.1: Scanning electron micrographs of the fifth abdominal segment of a *R. dominica* (a) male and (b) female; the arrow indicates the punctuate groove that is present in males but not in females

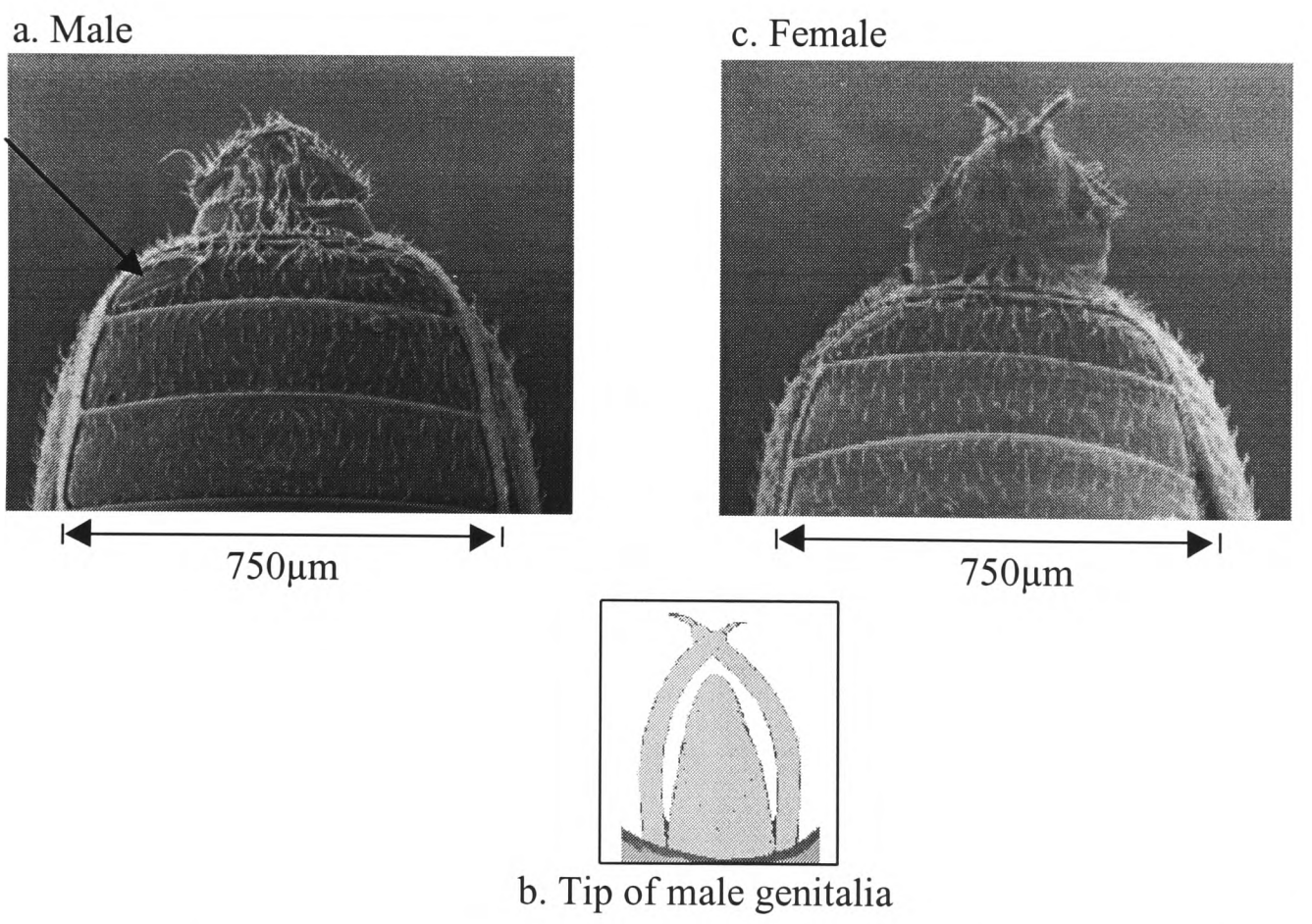


Figure 2.2: Scanning electron micrographs of the abdomen of a male and a female *R. dominica*; when the end of abdomen is gently squeezed, the tip of genitalia protrudes; (a) male genitalia are not apparent in the photograph but (b) can be seen in the diagram (Potter, 1935) (c) female genitalia can be clearly seen in the photograph

At the end of the experiment sexing was confirmed using the "squeezing method" of Crombie (1941) in which the tip of the abdomen of the adults is gently squeezed until the genitalia appear which can then be examined under a microscope (Figure 2.2).

2.4. OLFACTOMETER

2.4.1. Apparatus arrangement

The basic design of the olfactometer (Figure 2.3a, b & c) was similar to that used by Pettersson (1970). A four-pointed star-shaped exposure chamber was milled into an aluminium plate (30 x 30 x 1.2 cm), with a hole (8 mm diameter) drilled into the walls at each point. A glass plate (30 x 30 x 0.6cm) served as the floor and another glass plate, of the same size but with a hole (8 mm diameter) in its centre, served as a cover. Since *R. dominica* cannot walk on smooth surfaces a sheet of plain white paper (Dudley Stationery Ltd., England) was used as a floor covering. A small aluminium pipe (10 mm long, 8 mm outer / 6 mm inner diameter) was fixed in the central hole of the glass cover. Aluminium pipes (60 mm length, 8 mm outer / 6 mm inner diameter) were inserted through the holes of the chamber walls so that the pipes extended into the arena for 5 mm. The olfactometer was housed in a CTH room running at 27°C, 60% r.h. and 12 hour light/dark cycle with no natural light and was illuminated by fluorescent tubes. The beetles were always tested between 10.00 h and 16.00 h.

The air stream through the olfactometer was supplied by a vacuum pump (DA7C; Charles Austen Pumps Ltd., England) through 8 mm inner diameter polythene tubing (Fisher Scientific, UK). Immediately after the pump (Figure 2.3a), the air

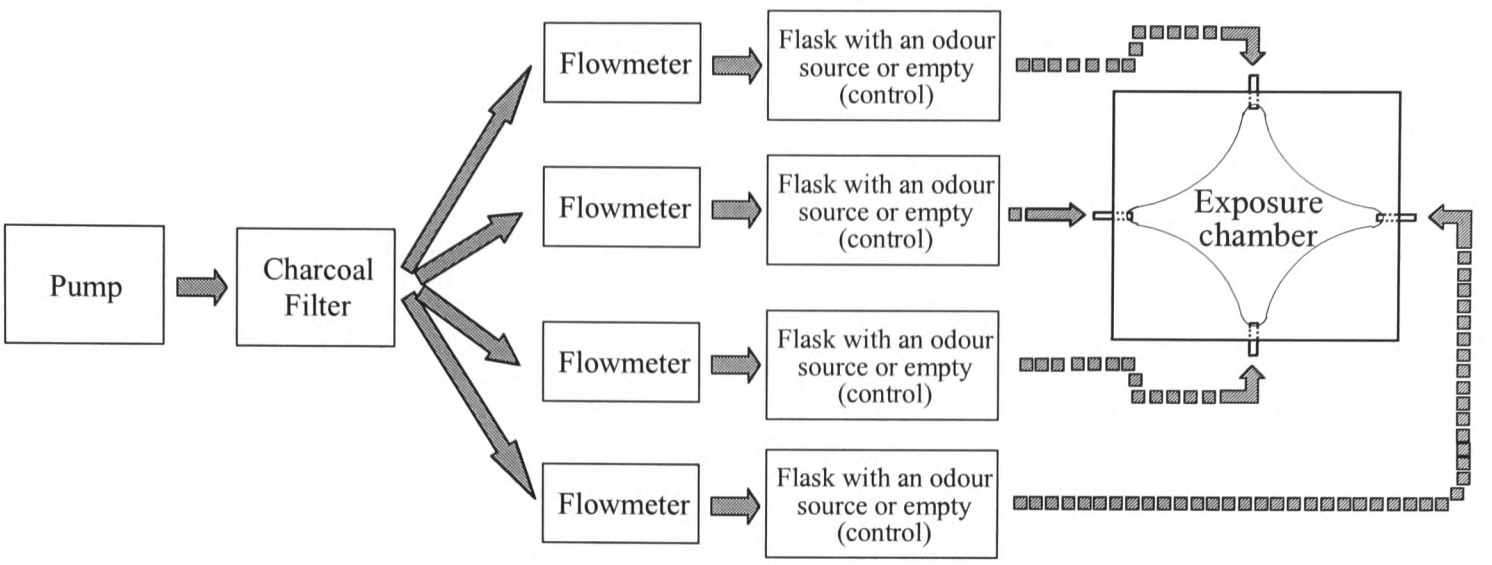


Figure 2.3a: Bioassay apparatus arrangement (arrows indicate direction of airflow)

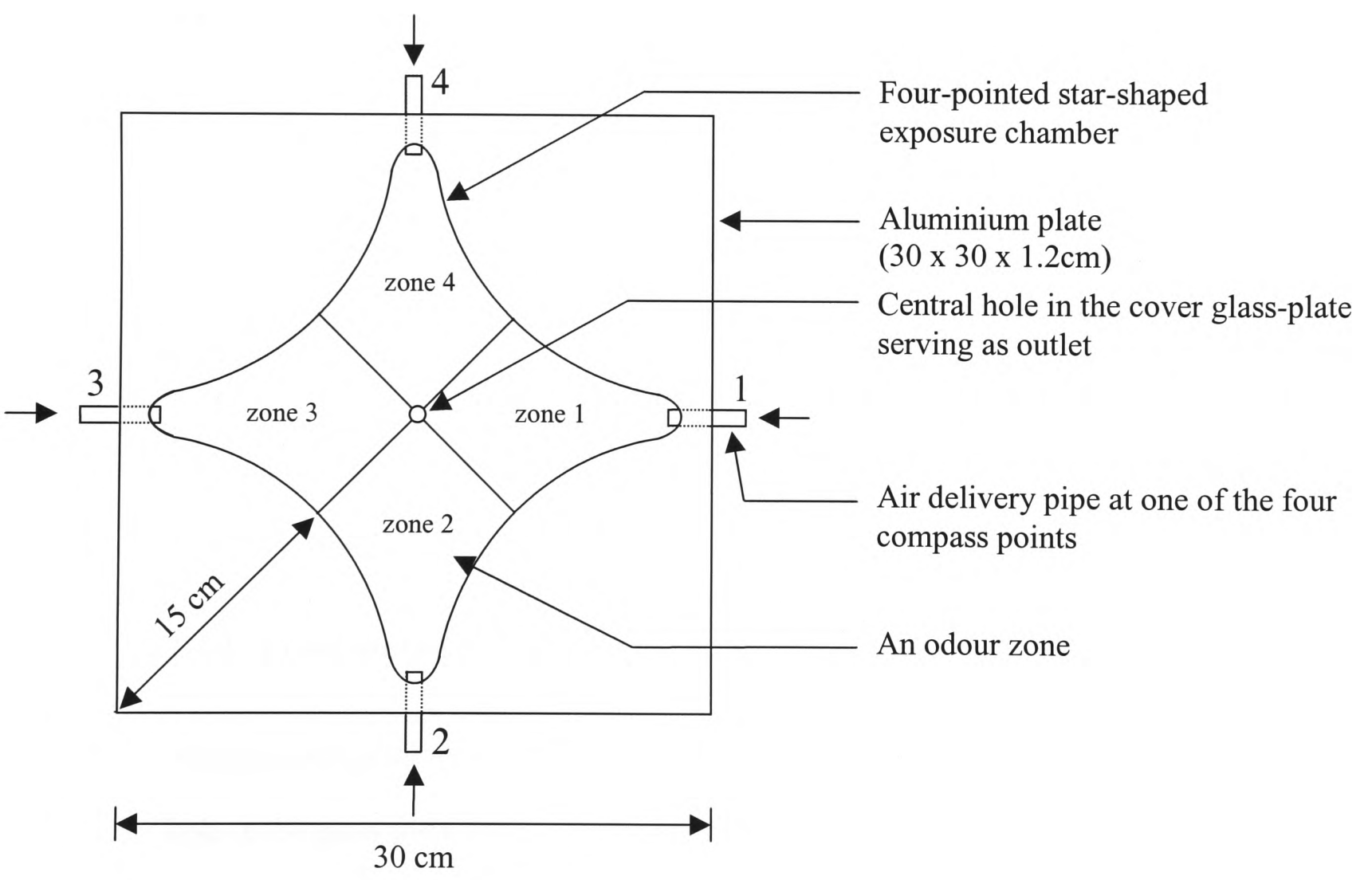


Figure 2.3b: Plan view of the four-choice airflow olfactometer exposure chamber

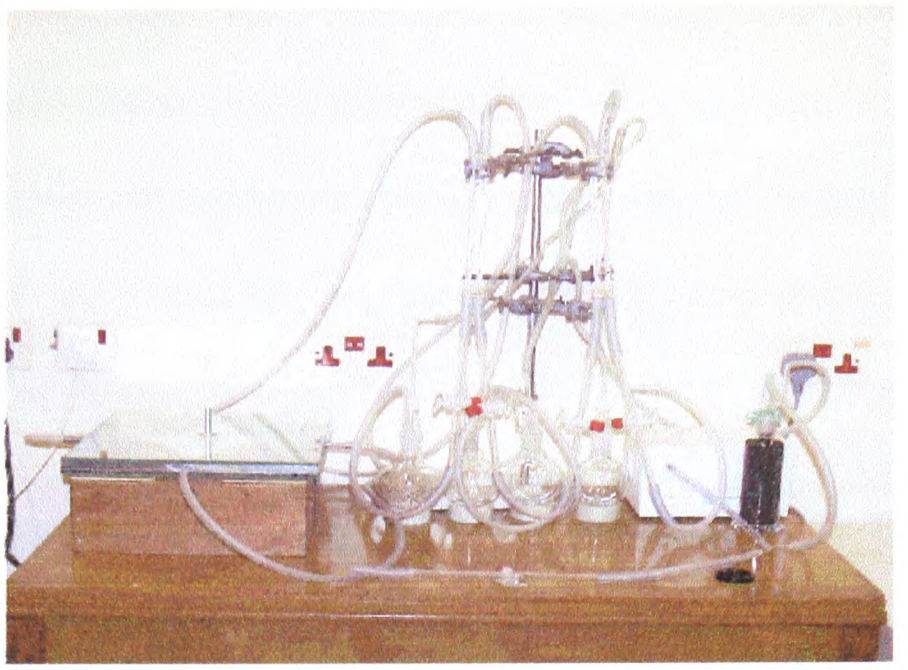


Figure 2.3c: Photograph of bioassay apparatus used to observe responses of adult *R. dominica* males and females to different odour sources

moved through a glass jar filled with activated charcoal to clean it. This air stream was then divided and pushed through four flowmeters (D1X 640, Meterate GPE, England). Each air stream then passed through a glass round-bottomed flask (1 litre), which contained either a volatile source or was empty and served as control. From each flask, air was delivered into the bioassay exposure chamber at one of the four (compass) points. The rate of airflow through each pipe was 250 cm^3 per minute. The air escaped from the chamber through the central hole in the cover glass plate. The air streams formed four distinct zones in the chamber (tested using a Draeger smoke generator).

2.4.2. Experimental procedure

A single test beetle was released into the centre of the chamber through the central hole in the glass-plate cover. To increase the chances that recorded beetle response was due to choice of a zone rather than movement caused by disturbance/handling, any beetles not spending ten seconds in the centre before moving into an odour

zone were not counted. Each beetle was observed for five minutes in the chamber and the time spent in different zones was recorded. When a beetle entered into one of the four air-delivery pipes, however, the test was terminated for that insect and the remaining time was awarded to that particular zone. The time spent by each beetle in each of the four odour zones was calculated to determine the preference of the beetles for different odour/volatile sources. Clean air served as control in all the cases except than when four odour sources were tested at a time.

One-odour source experiments

When one odour source was tested at a time, the volatiles were delivered into the exposure chamber from one compass point and from the other three compass points clean air was delivered.

Two-odour sources experiments

When volatiles from two odour sources were presented in the exposure chamber at the same time then the volatiles from those were always delivered from the opposite compass points. Clean air was delivered from the other two compass points.

Multiple-odour sources experiments

When more than two odour sources were tested at a time, the compass points were assigned randomly to different odour sources. Clean air was delivered from the fourth compass point when three odour sources were tested.

In all the experiments, after testing every quarter of the beetles, (after every $^{1}/_{4}$ olfactometer tests), the order in which the volatile delivery tubes were connected to

the exposure chamber was changed such that each zone of the test arena received volatiles from each source during the experiment. The sheet of paper used as covering of floor glass plate of exposure chamber to facilitate *R. dominica* walking was changed after testing every ten insects, unless stated otherwise in specific experiments.

2.5. PHEROMONE ENTRAINMENT

2.5.1. Equipment and their arrangement

The pheromone collection apparatus arrangement is illustrated in Figure 2.4a and photographs of the apparatus are shown in Figure 2.4b and 2.4c. Porapak-Q filters with mesh size 50-80 (Chrompack, Netherlands) were used to collect the pheromone. The amount of Porapak-Q put in each filter was 200 mg. Air at a flow rate of one litre per minute and cleaned with activated charcoal was drawn through the pheromone entrainment chamber (2.5 cm inner diameter x 8 cm length), where possible pheromone sources i.e. male beetle or synthetic pheromone loaded polythene vial, was placed, and then through the Porapak-Q filter. A vacuum pump (DA7C; Charles Austen Pumps Ltd., UK) was used to draw air through the system. The pheromone was extracted from the filters by using three 0.50cm^3 aliquots of "distol" grade dichloromethane (Fisher Scientific, UK). The extract was then analysed by capillary gas chromatography (GC), this was done by Mr. Dudley Farman of Chemical Ecology Group, Pest Management Department, Natural Resources Institute, Chatham, UK.

Samples were analysed by using a fused silica capillary column (30 m x 0.32 mm; internal diameter) coated with CPWax52CB (Carbowax equivalent; Chrompack

UK) with helium carrier gas (0.5 kg cm^{-2}) and flame ionisation detection. Injection was splitless (1 µl) and the oven was temperature programmed at 60°C for 2 minutes then at 6°C $minutes^{-1}$ to 230°C. Data was captured and processed using EZChrom 6.8 software.

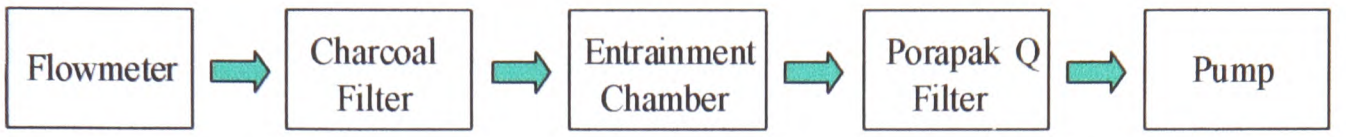


Figure 2.4a: Pheromone entrainment apparatus (arrows indicate direction of airflow)

Figure 2.4b: Entrainment apparatus used to collect pheromone released by adult *R. dominica* males; apparatus shown here is set to collect pheromone from two separate males

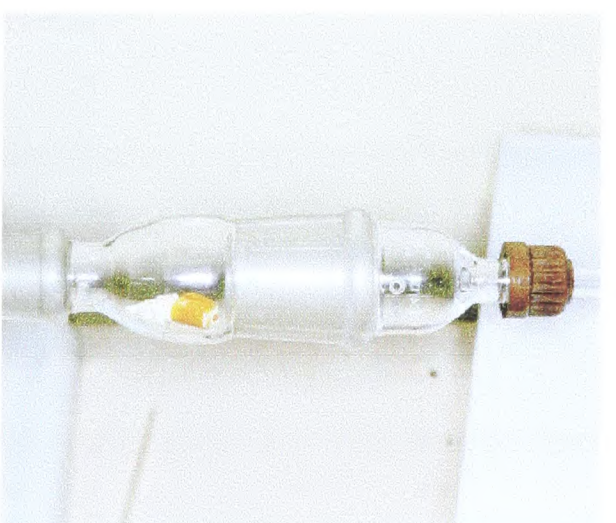


Figure 2.4c: Entrainment chamber containing a male *R. dominica* inside a maize grain

Under these conditions, D2 eluted at approximately 12.1 minutes, D1 at 12.2 minutes and the octyl acetate (5µg) used as internal standard at 12.5 minutes (Figure 2.5). The order of elution of the two pheromone components on this polar GC column is opposite to that on a non-polar GC column, as was used in the original identification work by Williams *et al.*, 1981. This was confirmed by analysis of the individual synthetic compounds and mass spectral analysis of both natural and synthetic compounds.

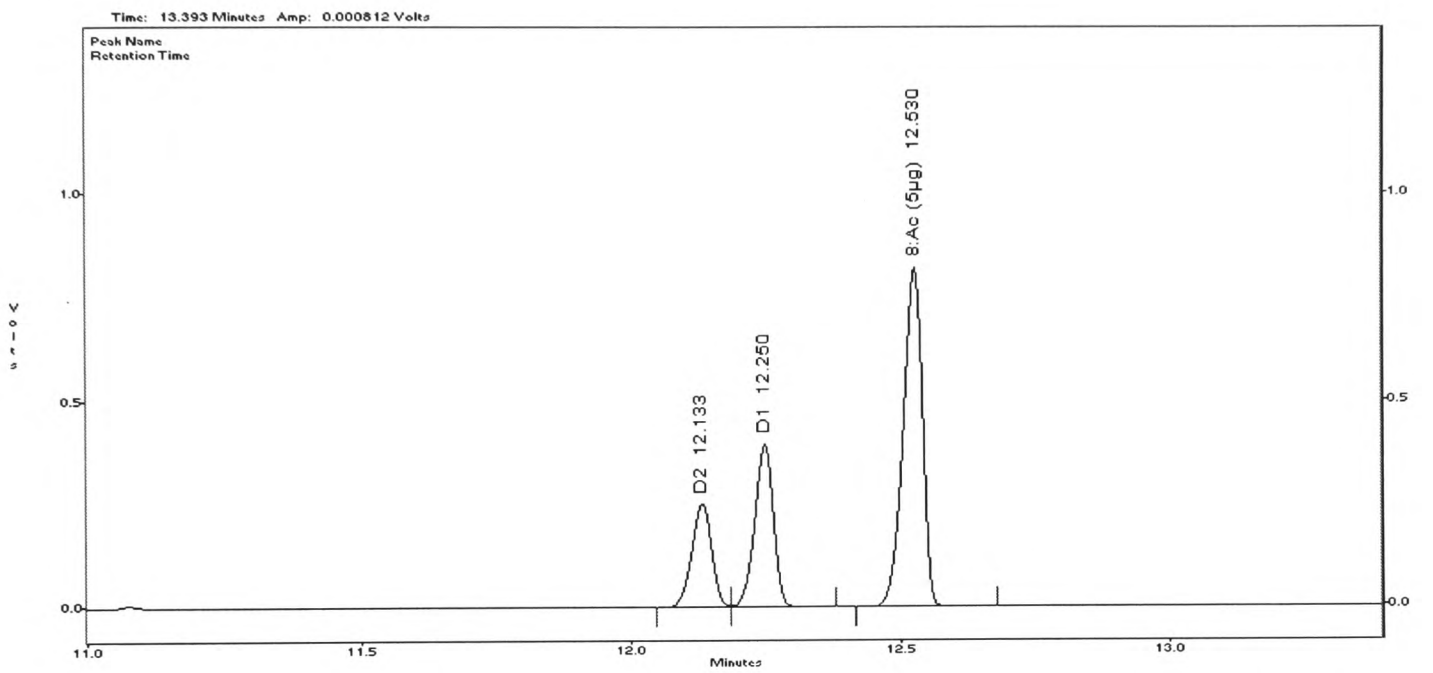


Figure 2.5: Capillary gas chromatogram showing peaks of D1, D2 and octyl acetate (internal standard)

2.5.2. Experimental procedure

The adult males of *R. dominica* used in pheromone entrainments were generally transferred singly, while still in a host-grain, into the entrainment chambers (Figure 2.4c) which has been flushed with clean air for twenty four hours before the collection of pheromone was started. The pheromone entrainments were generally made for a twenty-four-hour period, unless stated otherwise.

2.6. STATISTICAL ANALYSIS

Results were considered statistically significant when the probability of their occurrence by chance was less than five percent ($p \leq 0.05$).

2.6.1. Olfactometer experiments

The amount of time spent by each beetle in different odour zones of test arena was the parameter chosen for statistical analysis of a difference between odour sources (Figure 2.6).

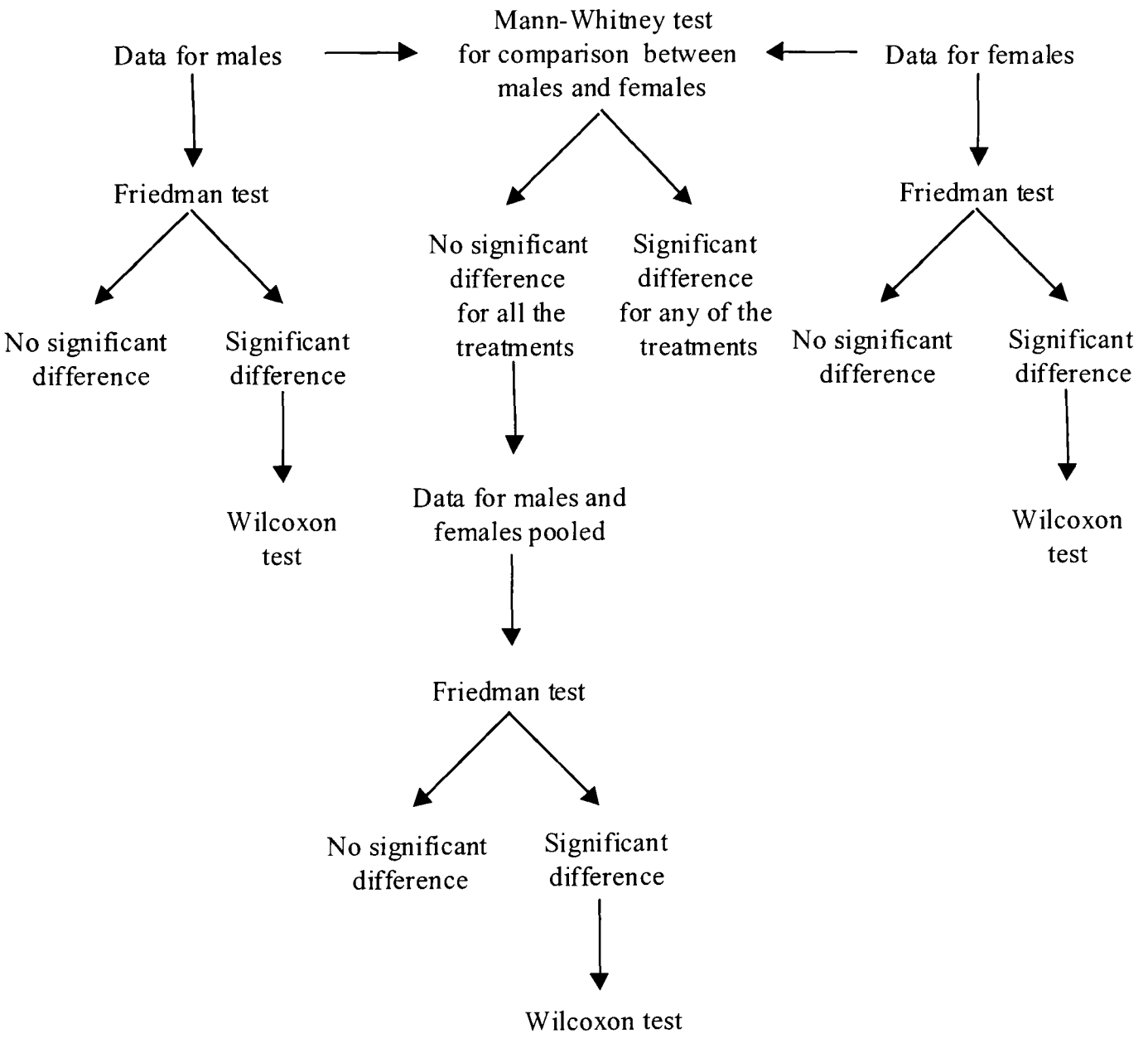


Figure 2.6: Scheme for statistical analysis of data recorded on behavioural responses of the adult *R. dominica* to different odour/volatile sources in olfactometer experiments

Friedman test for K-related samples was used to compare all the four sources. When the Friedman test showed a significant difference, a Wilcoxon test (for 2-related samples) was used for pairwise comparisons between sources. The responses of males and females were compared by a Mann-Whitney test for each of the four odour/volatile sources separately. The data for males and females were pooled when the Mann-Whitney test showed non-significant difference between males and females for all the odour/volatile sources. Friedman test for K-related samples was used to compare all the four sources. When the Friedman test showed a significant difference, a Wilcoxon test (for 2-related samples) was used for pairwise comparisons between sources. The responses of males and females were compared by a Mann-Whitney test for each of the four odour/volatile sources separately. The data for males and females were pooled when the Mann-Whitney test showed non-significant difference between males and females for all the odour/volatile sources.

Determination of level of response of males and females

The percentage of time spent by males or females in all the odour zones combined (excluding control), was calculated to determine the level of response. To investigate the difference between the level of response of males and females data were analysed using a Mann-Whitney test.

Determination of level of discrimination of males and females

To determine the level of discrimination of males or females between odour sources the following formula was applied. All the values obtained by subtracting

the response to one odour source from the response to other were considered as positive.

$$\text{Level of discrimination} = \frac{\text{Response to odour source 'A'} - \text{Response to odour source 'B'}}{\text{Response to odour source 'A'} + \text{Response to odour source 'B'}} \times 100$$

To determine the difference between the level of discrimination of males and females data were analysed using a Mann-Whitney test.

2.6.2. Pheromone entrainment experiments

When there were more than two treatments, one-way analysis of variance (ANOVA) was used to analyse the data from all the treatments while the Least Significant Difference (LSD) test was used for multiple comparisons between different treatments, unless stated otherwise.

Independent samples t-test was used to analyse the data from experiments with two treatments, unless stated otherwise.

Chapter: 3

Behavioural response of *R. dominica* adults to host-grain volatiles

3.1. INTRODUCTION

An important component of any model that attempts to quantify the early population dynamics of stored-grain insect pest populations is an accurate knowledge of the factors regulating the movement of the insects into and out of stores. Such factors are very complex, but life history parameters and presence or absence of aggregation pheromones and host volatiles are often important. The rate at which insects move into a grain storage facility is likely to depend, in part, on the ability of each species to use stimuli originating from the store (Dowdy *et al.*, 1993).

Phytophagous insects generally utilise volatile semiochemical cues from host plants during one or more phases of host selection (Phillips *et al.*, 1993). Many insect pests of field crops use plant volatiles as cues to find their hosts (Kainoh *et al.*, 1980; Nottingham and Coaker, 1985; Nottingham *et al.*, 1989; Mitchell *et al.*, 1991; Evans and Allen-Williams, 1993; Pivnick *et al.*, 1994). A number of bark beetle species also use host-plant volatiles to select suitable hosts (Byers *et al.*, 1985; Lanne *et al.*, 1987; Volz, 1988; Lindelow *et al.*, 1992; Macias-Samano *et al.*, 1998). Some insects are also able to discriminate between odours of host and non-host plants (Thiery and Visser, 1987; Kalinova *et al.*, 1996).

The process of selecting a suitable host in phytophagous insects consists of a

sequence of complex behavioural responses to stimuli associated with the host. A host may be rejected at any step of this process (Wood, 1982). Orientation of insects towards host odours is important, as it is often the first step in this process. Several stored-product insects are known to orient to stored-grain odours (Barrer & Jay, 1980; Freedman *et al.*, 1982; Barrer, 1983; Stubbs *et al.*, 1985; Pierce *et al.*, 1990; Phillips *et al.*, 1993). Host odours may be important for males of the insect species for whom presence of food is essential to release pheromones to attract females for mating (Landolt and Phillips, 1997). Such odours may also be important for females to locate oviposition sites (Crombie, 1941; Nottingham, 1988; Honda, 1995). *Rhyzopertha dominica* has been reported to show an orientation to wheat volatiles in laboratory experiments (Crombie, 1941; Dowdy *et al.*, 1993).

The current study aims to investigate the role of host volatiles in the primary selection of a food source in *R. dominica*. Behavioural responses of *R. dominica* towards different host volatiles were investigated to determine whether this insect could distinguish between the volatiles of one host (suitable) from others (less/unsuitable). Three host-grains, wheat [*Triticum vulgare* (L.)], maize [*Zea mays* (L.)] and groundnut (peanut) [*Arachis hypogaea* (L.)] were used as sources of volatiles. Wheat was considered to be the most suitable host for this particular strain of *R. dominica*, as a significantly greater number of F_1 adults emerged from wheat (551 from 5 pairs) than from maize (121 from 5 pairs) (Bashir, unpublished data). Maize was considered as an example of a moderately suitable host, as although the number of adults emerged on maize was much less than on wheat, the beetles were on average significantly heavier (61.43 mg/50 beetles) than those on wheat (60.64 mg/50 beetles). Groundnut was considered as an unsuitable or non-

host since *R. dominica* did not reproduce on it at all. However, the beetles were able to bore into the kernels and possibly also feed on them as they survived on groundnut kernels for approximately one month. This assessment of host suitability led to the hypothesis that *R. dominica* adults would show attraction to hosts in the order wheat, maize and groundnut.

Phytophagous insects may prefer a plant they have already experienced over one they have not experienced, whether or not this plant is more appropriate for their development (Bernays, 1995). This has been demonstrated for a few insect orders (De Boer and Hanson, 1984; Szentesi and Jermy, 1990) but the order Lepidoptera has been studied most extensively. Larvae of over twenty-four species of Lepidoptera have been shown to develop an altered preference in favour of the plant they have already experienced (Bernays, 1995). The cabbage looper moth, *Trichoplusia ni* (Hubner), learns the odour of a host plant when it first contacts the plant and subsequently shows attraction for that odour and not for others (Landolt and Monica, 1995). The effect of past experience on the behaviour of *R. dominica* in relation to host selection has not been studied. The present studies aimed to investigate the preference of *R. dominica* adults for different host-grain volatiles. The beetles used were reared on wheat and their behavioural responses to volatiles of wheat, maize and groundnut were observed. It seemed possible that the experiments were being biased by only investigating the responses of beetles reared on wheat. For this reason a study was made of the responses of beetles reared on two contrasting hosts– wheat and split green gram [*Vigna radiata* (L.)]. The beetles were reared on several alternative host grains for approximately one year, and the host-grain (split green gram) on which they did best was selected along with their most productive host-grain wheat to be used in this experiment.

3.2. MATERIALS AND METHODS

Olfactometer

Details of olfactometer apparatus and experimental procedures for recording observations of behavioural responses of beetles are given in section 2.4.

Statistical analysis

See section 2.6 for statistical methods used for analysis of data.

3.2.1. Response of beetles reared on one host-type to volatiles of another host-type

Insects

Beetles of known sex (see section 2.3) but unknown age were used in experiments using different odour sources.

Experimental procedure

Beetles reared on two commodities, wheat or split green gram, for approximately one year were used to test their response to volatiles from 500 g of wheat or split green gram. Volatiles of both the odour sources were presented in the exposure chamber at the same time (see section 2.4). One sample of each commodity was used in the experiment. Commodities were conditioned to the CTH room by leaving them there in a glass jar covered with muslin cloth for 24 hours before being used as odour sources. The responses of eighty beetles (40 males and 40 females) reared on wheat and eighty beetles (40 males and 40 females) reared on split green gram were recorded.

3.2.2. Comparative response of *R. dominica* adults to different host-grain volatiles

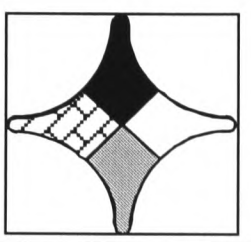
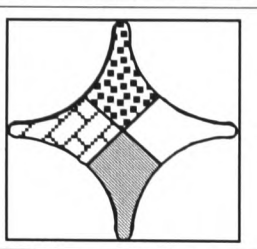
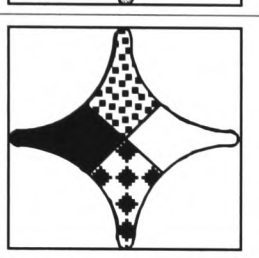
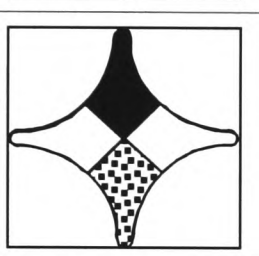
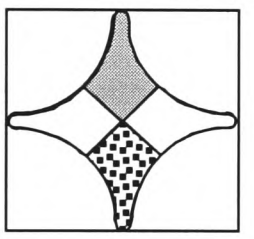
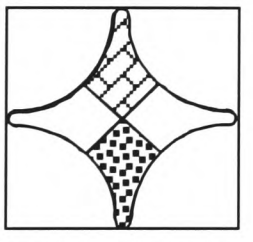
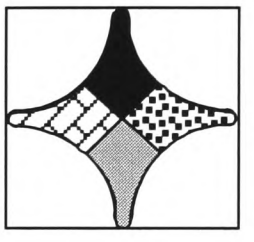
Insects

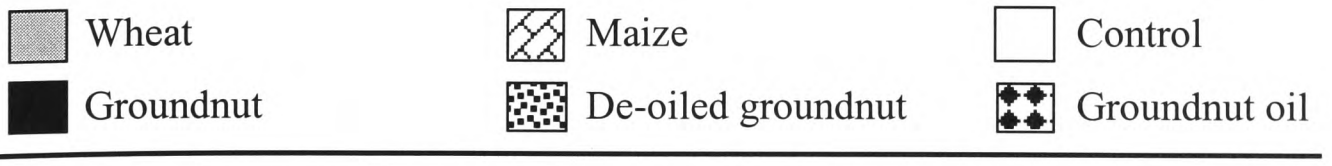
Beetles of known age and sex (see section 2.3) were used in the experiments using different odour sources.

Host-grain volatile sources and experimental procedure

Five sources of host volatiles, wheat, maize, groundnut, de-oiled groundnut and groundnut oil, were used in these studies (Figure 3.1). One sample of each odour source was used in any one experiment. The commodities were equilibrated to room temperature of 25±5°C for at least one week, and then for twenty-four hours to CTH room conditions set at 27±1°C and 60±5%r.h. before being used in olfactometer experiments. De-oiled groundnut was used in the experiments for the first time fourteen days after extraction of oil. The weight of wheat, maize or groundnut used as a volatile source was 125g. To ensure equivalence when using de-oiled groundnut or groundnut oil it was necessary to adjust the amount according to the measured oil content of the groundnut variety used in the test, which was 40%. Therefore, the olfactometer tests were undertaken with 75g of de-oiled groundnut (125g less 40%) or 50g of groundnut oil (the weight of oil in 125g of groundnut). Test (3.3.2.4) in which responses of beetles to groundnut and de-oiled groundnut were recorded was undertaken twice, the second occasion three months after the first. The same sample of de-oiled groundnut was re-tested to observe whether there was any change in host volatile output/beetle response with time.

Figure 3.1: Choice of different host-grain volatiles offered, and number of male and female beetles tested in different experiments

Test no.	Choice offered	No. of males tested	No. of females tested
3.3.2.1.		50	50
3.3.2.2.		20	20
3.3.2.3.		20	20
3.3.2.4.		20	20
3.3.2.5.		20	20
3.3.2.6.		20	20
3.3.2.7.		20	20



N.B. The numbers in the "Test no." column (e.g. 3.3.2.1.) refer to the heading numbers in the text.

De-oiling of Groundnut

Butt type extraction apparatus was used to extract the oil from groundnut kernels (Walker, 1979). Fifty grams of groundnut kernels were put in a thimble of known weight. Holes were drilled in the groundnut kernels using 1.5 mm diameter drill for easier extraction of the oil. The thimble was plugged with cotton wool and placed in the extraction apparatus. About 120 ml of the solvent, petroleum ether, was poured into 250 ml-glass flask of known weight. A few anti-bumping granules (BDH Ltd., UK), were added to the flask and then it was fixed to the extraction apparatus on an electric heating bath. The flask was heated to approximately 50-60^{o}C so that the solvent boiled moderately. After eight hours, the heating was stopped and the apparatus was allowed to cool down. The flask was removed from the apparatus and the oil was separated from the solvent by evaporating the solvent in a rotary evaporator. The flask containing oil was then put in the electric oven for two hours to remove the last traces of the solvent. Then the flask containing oil was weighed and the weight of oil was determined by subtracting the weight of flask from that of flask plus oil. The whole process was repeated using the same kernels to make sure that all the oil had been extracted.

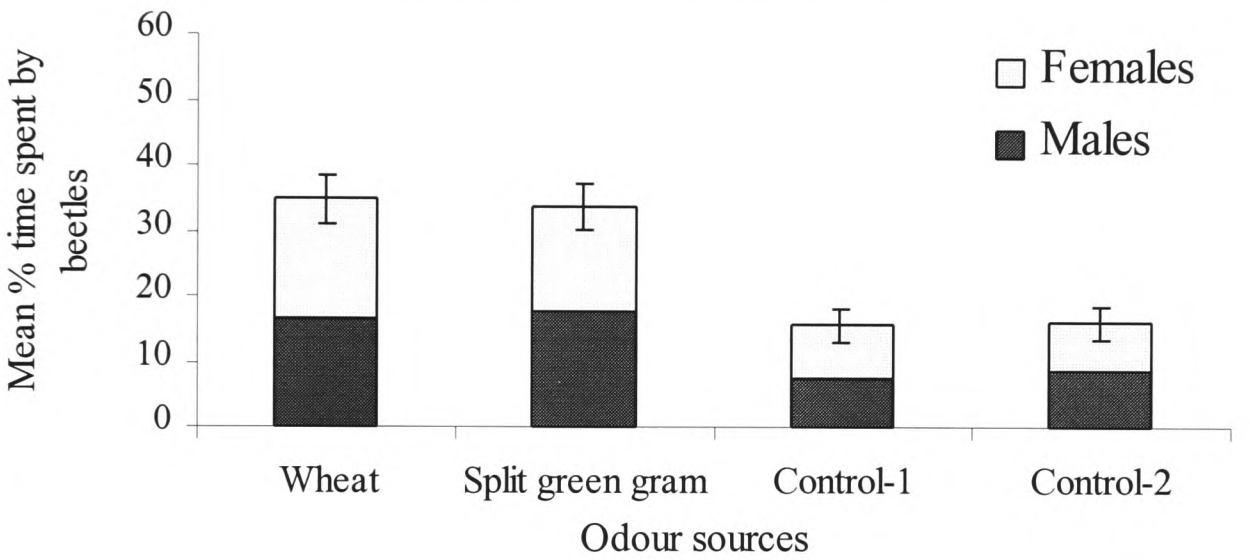
To check that a full oil extraction from whole kernels was being achieved an extraction was also undertaken on kernels after they had been ground/crushed into a coarse flour.

3.3. RESULTS

3.3.1. Response of beetles reared on one host-type to volatiles of another host-type

The beetles reared on either of the host-grains, wheat or split green gram, spent almost equal time in the zones of test arena receiving wheat or split green gram volatiles (Figure 3.2a & 3.2b).

a. Responses of beetles reared on wheat



b. Responses of beetles reared on split green gram

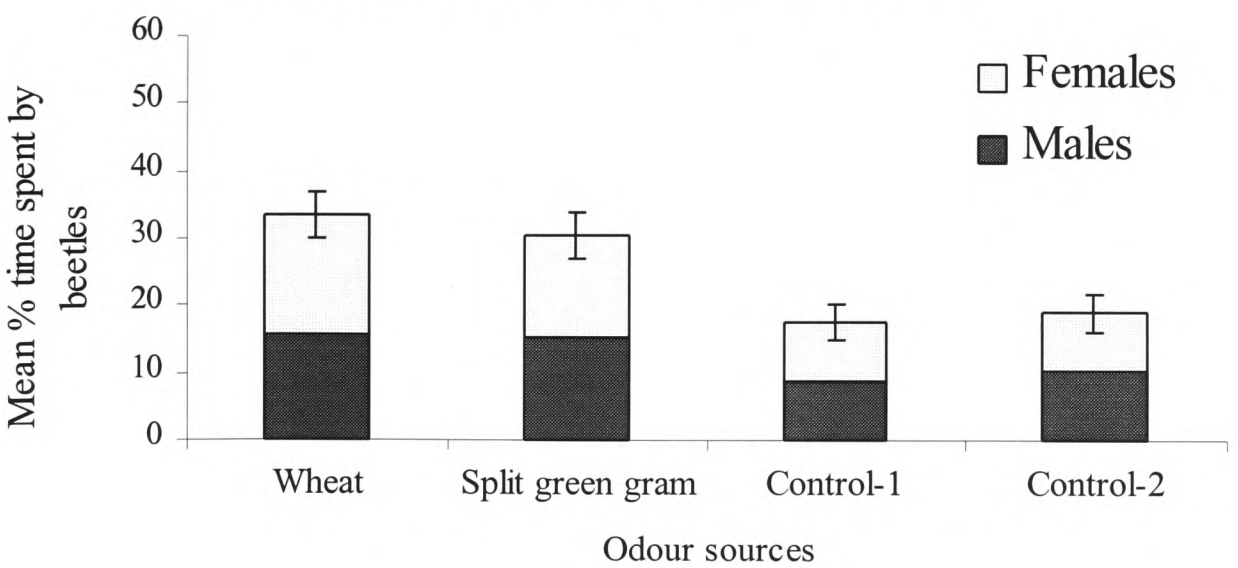


Figure 3.2: Mean percentage of the time (5 minutes) spent by adult *R. dominica* in different odour zones of a four choice airflow olfactometer receiving wheat or split green gram volatiles; error bars represent the standard error for males and females combined (n=80)

There was significant heterogeneity between treatments for males and for females in both, beetles reared on wheat or beetles reared on split green gram (Table-3.1). However, subsequent pairwise comparisons between different treatments demonstrated statistically significant differences only between the odour sources and controls (Table-3.2). This prompted analysis of pooled data from males and females, as there was no significant difference in response between males and females for wheat ($z=-0.33$, $p=0.74$, $n=40$), split green gram ($z=-0.57$, $p=0.57$, $n=40$), control-1 ($z=-0.26$, $p=0.80$, $n=40$) and control-2 ($z=-0.04$, $p=0.97$, $n=40$) for beetles reared on wheat, and for treatments, wheat ($z=-0.44$, $p=0.66$, $n=40$), split green gram ($z=-0.00$, $p=1.00$, $n=40$), control-1 ($z=-0.35$, $p=0.73$, $n=40$) and control-2 ($z=-0.17$, $p=0.86$) for beetles reared on split green gram.

As expected the pooled data showed significant heterogeneity among treatments (Table-3.1) but subsequent pairwise comparison once again demonstrated statistically significant differences only between the odour sources and controls (Table-3.2).

Table-3.1: Statistical comparison of the response of *R. dominica* adults to different treatments, on the basis of percentage of time spent by beetles in different zones of a four-choice airflow olfactometer; data were analysed using Friedman test for K-related samples

	Beetles reared on wheat			Beetles reared on split green gram		
	Chi-Square	df	p-value	Chi-Square	df	p-value
Males	11.67	3	<0.01	8.22	3	0.04
Females	11.60	3	<0.01	9.12	3	0.03
Pooled	22.65	3	<0.001	17.14	3	<0.001

Table-3.2: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from wheat, split green gram or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females		Pooled	
	Z	p-value	Z	p-value	Z	p-value
a. Beetles reared on wheat						
Wheat *vs.* SGG*	-0.46	0.64	-0.34	0.74	-0.09	0.93
Wheat *vs.* Control-1	-2.23	0.03	-2.61	<0.01	-3.55	<0.001
Wheat *vs.* Control-2	-2.35	0.02	-2.90	<0.01	-3.74	<0.001
SGG *vs.* Control-1	-2.87	<0.01	-2.43	0.02	-3.66	<0.001
SGG *vs.* Control-2	-2.36	0.02	-2.41	0.02	-3.47	<0.001
Control-1 *vs.* Control-2	-0.21	0.83	-0.35	0.73	-0.340	0.69
b. Beetles reared on split green gram						
Wheat *vs.* SGG	-0.40	0.69	-0.48	0.63	-0.62	0.54
Wheat *vs.* Control-1	-2.20	0.03	-2.29	0.02	-3.15	<0.01
Wheat *vs.* Control-2	-1.44	0.15	-2.34	0.02	-2.77	<0.01
SGG *vs.* Control-1	-1.79	0.07	-2.07	0.04	-2.73	<0.01
SGG *vs.* Control-2	-1.57	0.02	-1.20	0.06	-2.56	<0.01
Control-1 *vs.* Control-2	-0.42	0.67	-0.17	0.87	-0.43	0.67

NB. SGG* = Split green gram

3.3.2. Comparative response of *R. dominica* adults to different host-grain volatiles

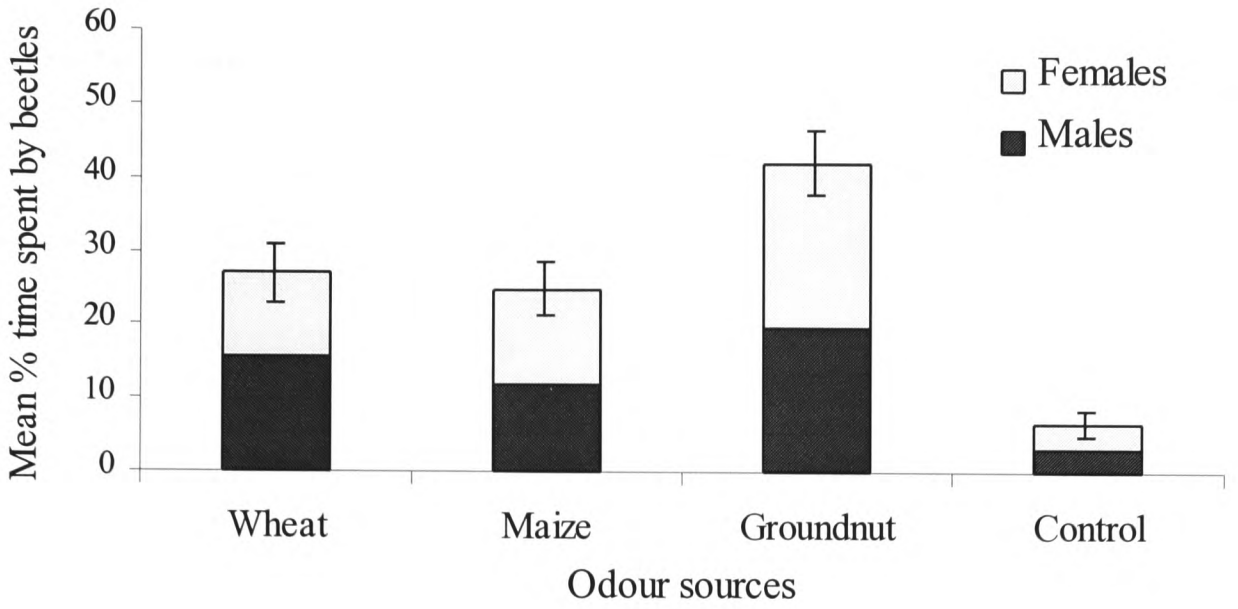
3.3.2.1. Wheat, maize and groundnut

3.3.2.2. Wheat, maize and de-oiled groundnut

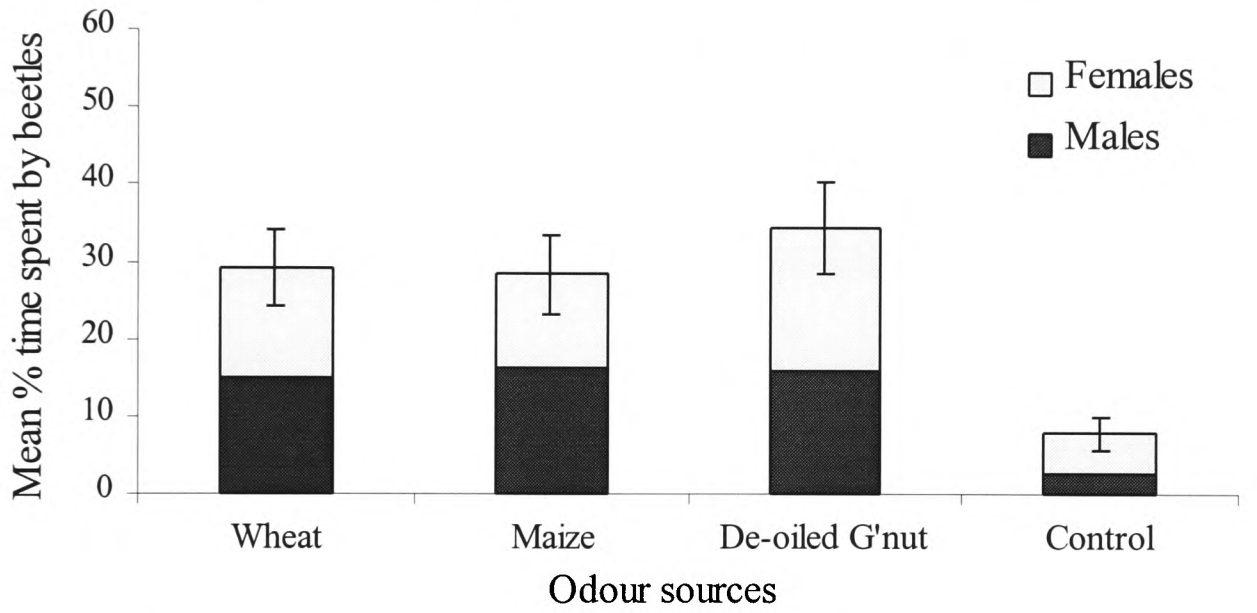
3.3.2.3. Groundnut, de-oiled groundnut and groundnut oil

Adult *R. dominica* spent more time in the zone receiving groundnut volatiles than in either of the zones receiving wheat or maize volatiles when the choice among these three was offered (Figure 3.3a).

a. Preference among wheat, maize and groundnut (n=100)



b. Preference among wheat, maize and de-oiled groundnut (n=40)



c. Preference among groundnut, de-oiled groundnut and groundnut oil (n=40)

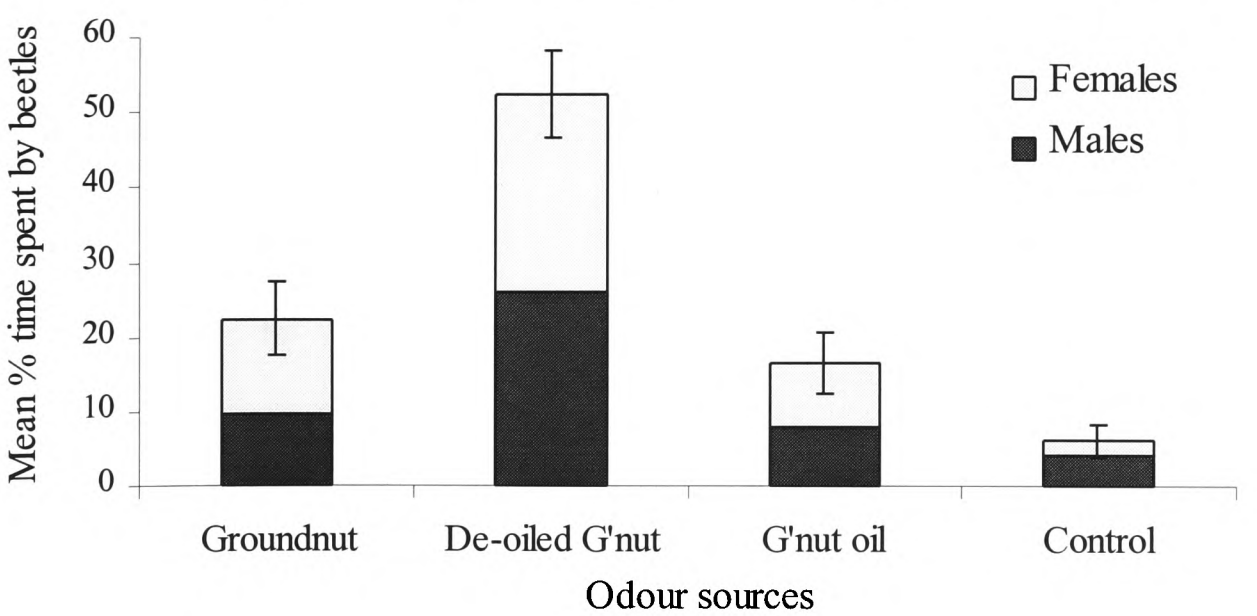


Figure 3.3: Mean percentage of the time (5 minutes) spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer; error bars represent the standard error for males and females combined

There was significant heterogeneity among treatments for males and females, and data for males and females pooled (Table-3.3a) as there was no significant difference between males and females for any of the treatments [wheat ($z=-0.98$, $p=0.33$, $n=50$), maize ($z=-0.56$, $p=0.58$, $n=50$), groundnut ($z=-0.81$, $p=0.42$, $n=50$) and control ($z=-0.63$, $p=0.53$, $n=50$)].

Subsequent pairwise comparisons between different treatments for males and females demonstrated statistically significant difference only between the odour sources and control (Table-3.4a). However, comparisons for pooled data demonstrated a significantly greater positive response of beetles to groundnut volatiles than to maize or wheat volatiles (Table-3.4a).

These results were opposite to initial hypothesis that suitable host would be more attractive i.e. the beetles would prefer wheat or maize over groundnut. The beetles were attracted more strongly towards groundnut volatiles than wheat or maize volatiles. It seemed possible that this strong positive response could be due to the oil content of the groundnut. To test this possibility the attractiveness of wheat and of maize volatiles was compared with those from de-oiled groundnut kernels.

When the choice among the volatiles of wheat, maize and de-oiled groundnut was offered, the females spent longer in the zone receiving de-oiled groundnut volatiles but males spent nearly equal time in the zones of the test arena receiving volatiles from any of the odour sources (Figure 3.3b). Statistical analysis showed significant difference among treatments for males but not for females (Table-3.3b). However, analysis of data for males and females pooled did show significant difference among treatments (Table-3.3b). The analysis of pooled data was undertaken after no significant difference was found between males and females for wheat ($z=-0.10$,

p=0.93, n=20), maize (z=-0.95, p=0.36, n=20), de-oiled groundnut (z=-0.32, p=0.76, n=20) and control (z=-0.91, p=0.45, n=20).

Table-3.3: Statistical comparison of the response of *R. dominica* adults to different treatments, on the basis of percentage of time spent by beetles in different zones of a four-choice airflow olfactometer; data were analysed using Friedman test for K-related samples

	Chi-Square	df	p-value
a. Wheat, maize and groundnut			
Males	12.73	3	<0.01
Females	19.13	3	<0.001
Pooled	34.47	3	<0.001
b. Wheat, maize and de-oiled groundnut			
Males	11.03	3	<0.01
Females	2.90	3	0.41
Pooled	11.77	3	<0.01
c. Groundnut, de-oiled groundnut and groundnut oil			
Males	13.94	3	<0.01
Females	19.30	3	<0.001
Pooled	30.74	3	<0.001

Pairwise comparisons between treatments for males and pooled data demonstrated significant differences only between odour sources and control (Table-3.4b).

The percentage of time beetles (males + females) spent in the zone of the test arena receiving de-oiled groundnut volatiles in this experiment was smaller (35%) than the time spent in the zone receiving groundnut volatiles in the previous experiment (42%), when choice among wheat, maize and groundnut volatiles was offered.

This strengthened the possibility that attraction of *R. dominica* towards groundnut was due to oil content. To confirm this, the beetles were offered the choice among volatiles of groundnut, de-oiled groundnut and groundnut oil. The results demonstrated that the beetles showed much greater positive response to de-oiled groundnut volatiles compared to groundnut or groundnut oil volatiles (Figure 3.3c).

Statistical analysis showed treatments differed significantly for both males and females (Table-3.3c). A non-significant difference between the responses of males and females for all the treatments, groundnut ($z=-0.45$, $p=0.69$, $n=20$), de-oiled groundnut ($z=-0.18$, $p=0.86$, $n=20$), groundnut oil ($z=-0.04$, $p=0.97$, $n=20$) and control ($z=-1.61$, $p=0.18$, $n=20$), allowed the analysis of data for males and females pooled, and this also showed significant difference among treatments (Table-3.3c).

Pairwise comparisons demonstrated that males showed significantly greater positive response to volatiles from de-oiled groundnut than to those from either groundnut or groundnut oil (Table-3.4c). Female response to de-oiled groundnut was significantly greater than to groundnut oil but it was not significantly different from groundnut volatiles. Pairwise comparisons for pooled data showed significant differences between all the combinations except between groundnut and groundnut oil (Table-3.4c).

Table-3.4: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from different odour sources; data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females		Pooled	
	Z	p-value	Z	p-value	Z	p-value
a. Comparisons between wheat, maize and groundnut						
Wheat *vs.* Control	-3.41	<0.001	-2.35	0.02	-4.03	<0.001
Maize *vs.* Control	-2.98	<0.01	-2.68	<0.01	-3.97	<0.001
Groundnut *vs.* Control	-4.01	<0.001	-4.07	<0.001	-5.81	<0.001
Wheat *vs.* Maize	-0.80	0.43	-0.28	0.78	-0.32	0.75
Wheat *vs.* Groundnut	-0.74	0.46	-2.10	0.04	-2.04	0.04
Maize *vs.* Groundnut	-1.71	0.09	-1.52	0.13	-2.36	0.02
b. Comparisons between wheat, maize and de-oiled groundnut						
Wheat *vs.* Control	-2.58	<0.01	-2.43	0.02	-3.51	<0.001
Maize *vs.* Control	-3.41	<0.001	-1.19	0.23	-3.24	<0.001
D. G'nut* *vs.* Control	-2.40	0.02	-1.96	0.05	-3.10	<0.01
Wheat *vs.* Maize	-0.16	0.87	-0.52	0.60	-0.25	0.81
Wheat *vs.* D. G'nut	-0.28	0.78	-0.72	0.47	-0.75	0.46
Maize *vs.* D. G'nut	-0.19	0.85	-0.72	0.47	-0.43	0.67
c. Comparisons between groundnut, de-oiled groundnut and groundnut oil						
Groundnut *vs.* Control	-1.22	0.22	-2.85	<0.01	-2.94	<0.01
D.G'nut *vs.* Control	-3.40	<0.001	-3.63	<0.001	-4.79	<0.001
G'nut oil *vs.* Control	-1.07	0.29	-2.17	0.03	-2.21	0.03
Groundnut *vs.* D.G'nut	-2.42	0.02	-1.51	0.13	-2.70	<0.01
Groundnut *vs.* G'nut oil	-0.11	0.91	-1.14	0.26	-0.82	0.41
D. G'nut *vs.* G'nut oil	-2.52	<0.01	-2.36	0.02	-3.46	<0.001

NB. D. G'nut* = De-oiled Groundnut

3.3.2.4. Groundnut and de-oiled groundnut

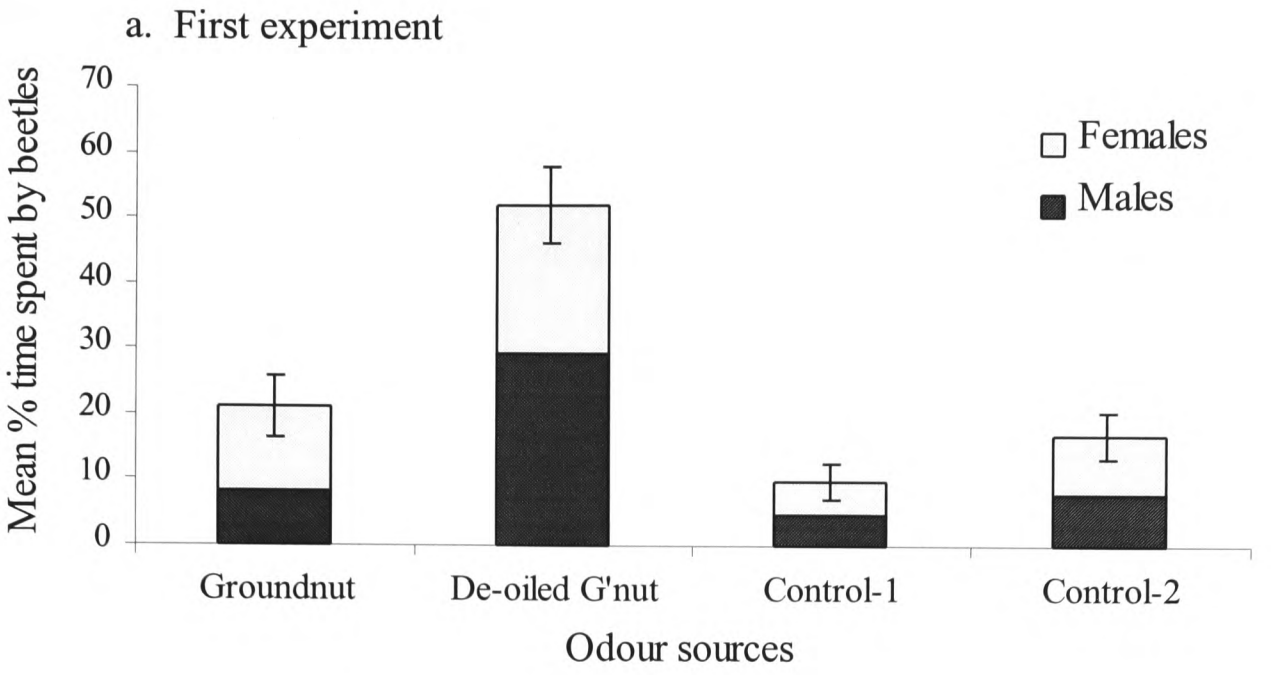
3.3.2.5 Wheat and de-oiled groundnut

3.3.2.6. Maize and de-oiled groundnut

In earlier experiments, volatiles of three odour sources were delivered in the olfactometer exposure chamber simultaneously. To remove suspicions that the presentation of volatiles from too many sources at the same time could be masking the response to each other, pairwise comparisons between de-oiled groundnut and groundnut, wheat or maize were made.

The beetles spent considerably more time in the zone receiving de-oiled groundnut volatiles than in that receiving groundnut volatiles, in two experiments (Figure 3.4). Statistical analysis showed significant heterogeneity among treatments in both the cases for both males and females (Table-3.5). Analysis of the data for males and females pooled also showed significant difference among treatments. This being possible as there was no significant difference between the resposnes of males and females for different treatments in both the experiments. [First experiment; groundnut ($z=-0.34$, $p=0.76$, $n=20$), de-oiled groundnut ($z=-1.17$, $p=0.25$, $n=20$), control-1 ($z=-0.81$, $p=0.46$, $n=20$) and control-2 ($z=-0.38$, $p=0.72$, $n=20$): Second experiment; groundnut ($z=-0.97$, $p=0.36$, $n=20$), de-oiled groundnut ($z=-0.34$, $p=0.76$, $n=20$), control-1 ($z=-0.29$, $p=0.86$, $n=20$) and control-2 ($z=-0.83$, $p=0.62$, $n=20$)].

Pairwise comparisons for either of the experiments, demonstrated significant difference between the responses to groundnut volatiles and de-oiled groundnut volatiles only for males in the first experiment (Table-3.6).



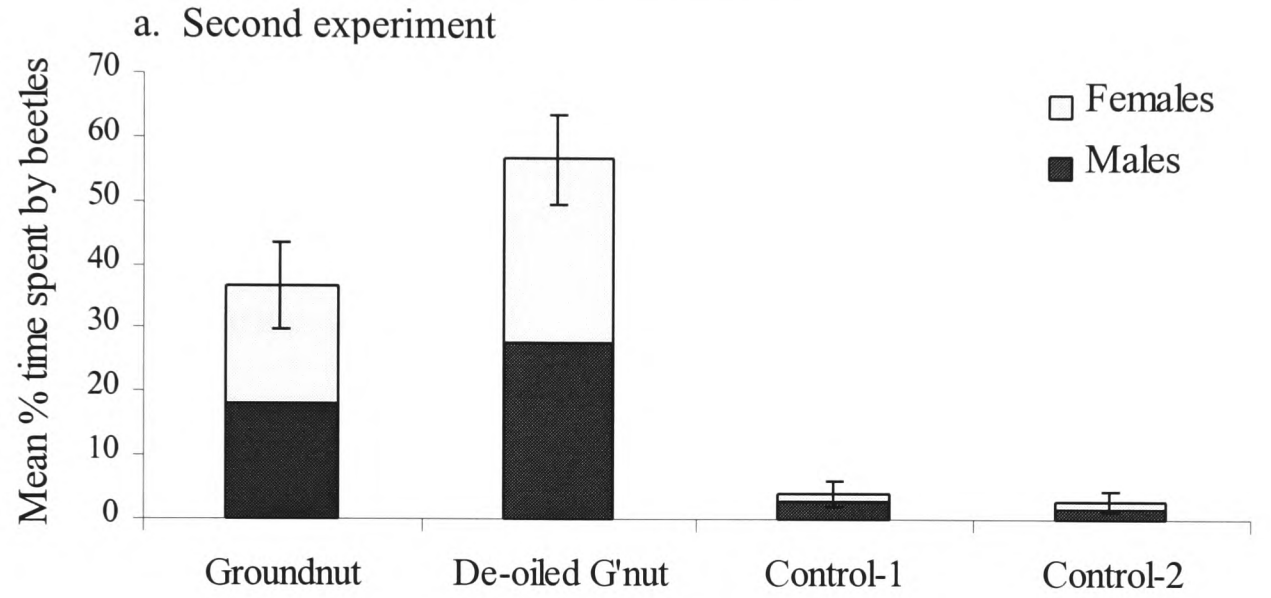


Figure 3.4: Mean percentage of the time spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer; second experiment was undertaken three months after the first; error bars represent the standard error for males and females combined (n=40)

Table-3.5: Statistical comparison of the response of *R. dominica* adults to different treatments, on the basis of percentage of time spent by beetles in different zones of a four-choice airflow olfactometer; data were analysed using Friedman test for K-related samples

	First experiment			Second experiment		
	Chi-Square	df	p-value	Chi-Square	df	p-value
Males	19.99	3	<0.001	14.91	3	<0.01
Females	7.97	3	0.047	33.49	3	<0.001
Pooled	24.08	3	<0.001	46.90	3	<0.001

Table-3.6: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from groundnut, de-oiled groundnut or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females		Pooled	
	Z	p-value	Z	p-value	Z	p-value
a. First experiment						
Groundnut *vs.* D. G'nut*	-2.88	<0.01	-1.23	0.22	-2.80	<0.01
Groundnut *vs.* Control-1	-1.16	0.25	-1.63	0.10	-2.00	0.045
Groundnut *vs.* Control-2	-0.04	0.97	-0.52	0.61	-0.49	0.63
D. G'nut *vs.* Control-1	-3.42	<0.001	-2.59	<0.01	-4.34	<0.001
D. G'nut *vs.* Control-2	-2.88	<0.01	-1.92	0.02	-3.56	<0.001
Control-1 *vs.* Control-2	-0.47	0.64	-1.19	0.23	-1.42	0.16
b. Second experiment						
Groundnut *vs.* D. G'nut	-1.07	0.29	-0.91	0.37	-1.30	0.19
Groundnut *vs.* Control-1	-2.34	0.02	-3.02	<0.01	-3.76	<0.001
Groundnut *vs.* Control-2	-2.43	0.02	-3.10	<0.01	-3.95	<0.001
D. G'nut *vs.* Control-1	-3.07	<0.01	-3.53	<0.001	-4.61	<0.001
D. G'nut *vs.* Control-2	-3.20	<0.001	-3.53	<0.001	-4.67	<0.001
Control-1 *vs.* Control-2	-0.73	0.47	-0.41	0.69	-0.00	0.44

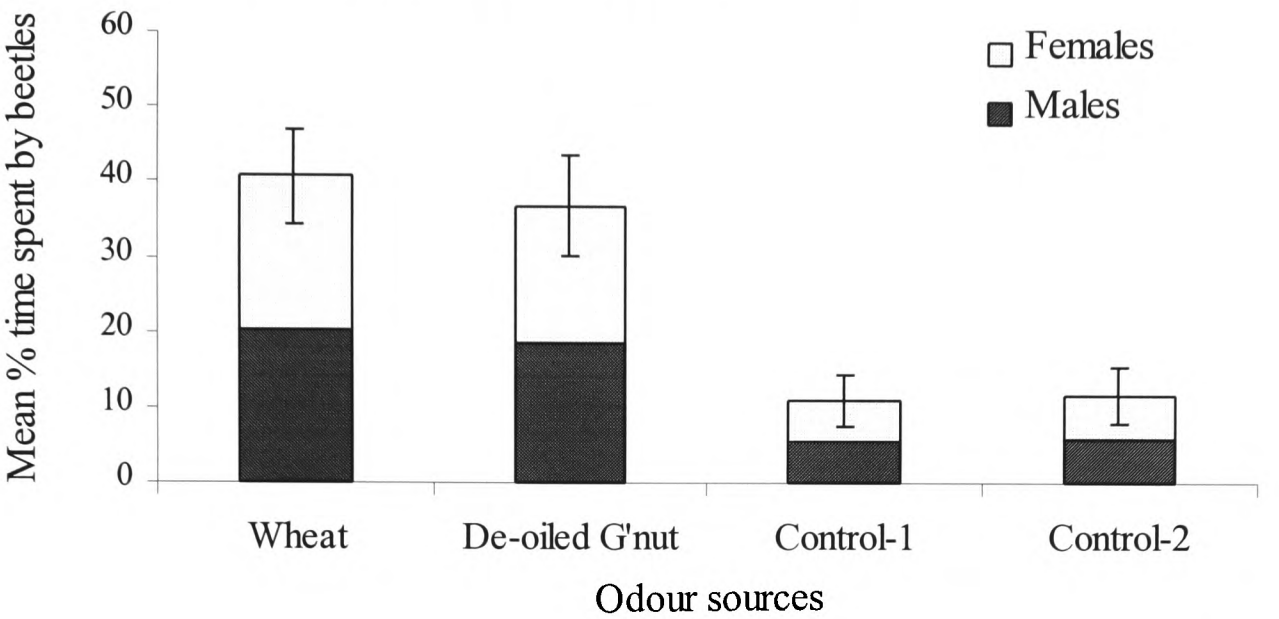
NB. D. G'nut* = De-oiled Groundnut

When the attractiveness of de-oiled groundnut volatiles was compared with wheat, or maize volatiles, the beetles showed similar response to the odour sources, in both the experiments (Figure 3.5).

There was significant heterogeneity among treatments for females but not for males when de-oiled groundnut volatiles were tested against wheat volatiles (Table-3.7). However, there was significant difference among treatments for both males and

females when de-oiled groundnut was tested against maize (Table-3.7).

a. Preference between wheat and de-oiled groundnut



a. Preference between maize and de-oiled groundnut

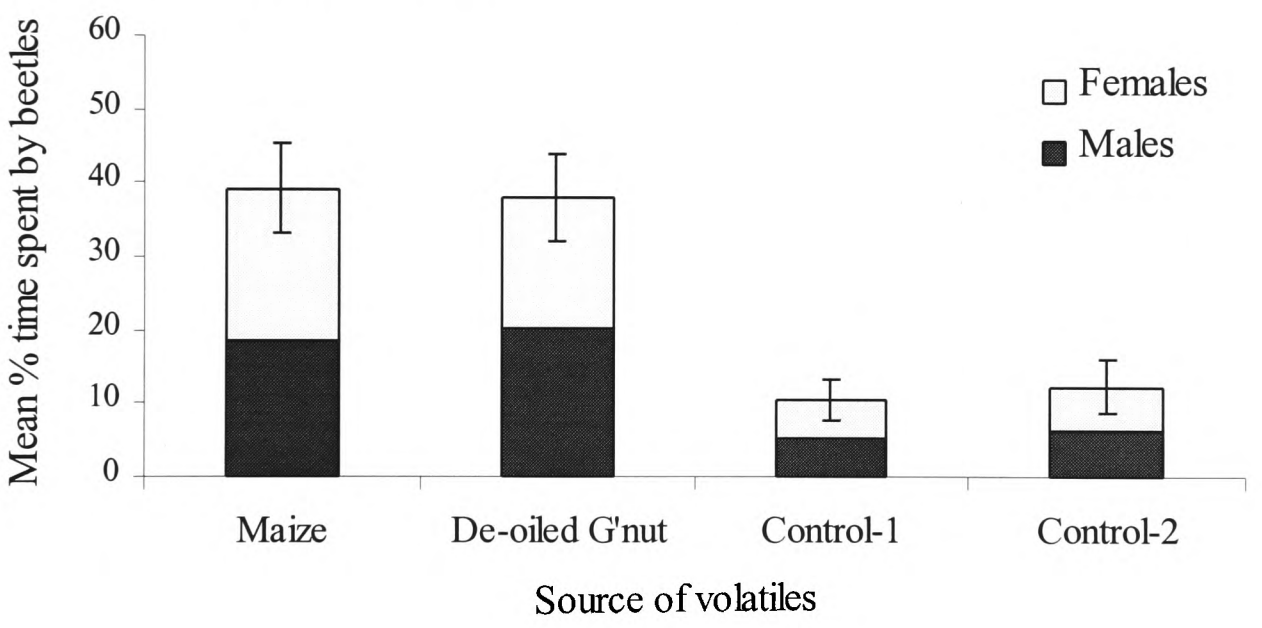


Figure 3.5: Mean percentage of the time (5 minutes) spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer; error bars represent the standard error for males and females combined (n=40)

NB. De-oiled G'nut = De-oiled Groundnut

Table-3.7: Statistical comparison of the response of *R. dominica* adults to different treatments, on the basis of percentage of time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from (A) wheat, de-oiled groundnut or clean air (control) or (B) maize, de-oiled groundnut or clean air (control); data were analysed using Friedman test for K-related samples

	(A)			(B)		
	Chi-Square	df	p-value	Chi-Square	df	p-value
Males	11.12	3	<0.01	8.75	3	0.03
Females	7.33	3	0.06	7.98	3	0.046
Pooled	17.90	3	<0.001	16.47	3	<0.001

There was no significant difference between male and female responses for any of the treatment in both the experiments. [wheat (z=-0.15, p=0.88, n=20), de-oiled groundnut (z=-0.26, p=0.82, n=20), control-1 (z=-0.55, p=0.66, n=20) and control-2 (z=-1.00, p=0.40, n=20)] [maize (z=-0.26, p=0.79, n=20), de-oiled groundnut (z=-0.33, p=0.74, n=20), control-1 (z=-0.32, p=0.75, n=20) and control-2 (z=-0.11, p=0.91, n=20)].

Data for males and females pooled, showed significant difference among treatments in both the experiments (Table-3.7). However, pairwise comparisons between treatments did not show significant difference between odour sources for males, females or pooled data in any of the experiments (Table-3.8).

Table-3.8: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from (a) wheat, de-oiled groundnut or clean air (control) or (b) maize, de-oiled groundnut or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females		Pooled	
	Z	p-value	Z	p-value	Z	p-value
a. Wheat and de-oiled groundnut						
Wheat *vs.* D. G'nut*	-0.04	0.97	-0.28	0.78	-0.20	0.84
Wheat *vs.* Control-1	-2.22	0.03	-2.62	<0.01	-3.36	<0.001
Wheat *vs.* Control-2	-2.30	0.02	-2.59	<0.01	-3.50	<0.001
D. G'nut *vs.* Control-1	-1.95	0.05	-2.17	0.03	-2.81	<0.01
D. G'nut *vs.* Control-2	-2.12	0.03	-1.99	0.046	-2.83	<0.01
Control-1 *vs.* Control-2	-0.28	0.78	-0.13	0.89	-0.22	0.83
b. Maize and de-oiled groundnut						
Maize *vs.* D. G'nut	-0.32	0.75	-0.63	0.53	-0.71	0.48
Maize *vs.* Control-1	-2.42	0.02	-2.35	0.02	-3.36	<0.001
Maize *vs.* Control-2	-2.20	0.03	-2.52	<0.01	-3.39	<0.001
D. G'nut *vs.* Control-1	-2.28	0.02	-2.67	<0.01	-3.53	<0.001
D. G'nut *vs.* Control-2	-2.02	0.04	-2.17	0.03	-2.98	<0.01
Control-1 *vs.* Control-2	-0.16	0.88	-0.42	0.68	-0.20	0.85

NB. D. G'nut* = De-oiled Groundnut

3.3.2.7. Wheat, maize, groundnut and de-oiled groundnut

The beetles spent nearly the same time in all four odour zones of test arena when their responses to volatiles of four host-grains were tested simultaneously (Figure 3.6).

There was no significant difference between treatments for males (Chi-

Square=2.95, df=3, p=0.40), females (Chi-Square=0.70, df=3, p=0.87) and for pooled data (Chi-Square=1.73, df=3, p=0.63, n=20). The analysis of the pooled data from males and females was possible as there was no significant difference in response between males and females for wheat (z=0.00, p=1.00, n=20), maize (z=-0.24, p=0.82, n=20), groundnut (z=-0.79, p=0.45, n=20) and de-oiled groundnut (z=-0.78, p=0.45, n=20).

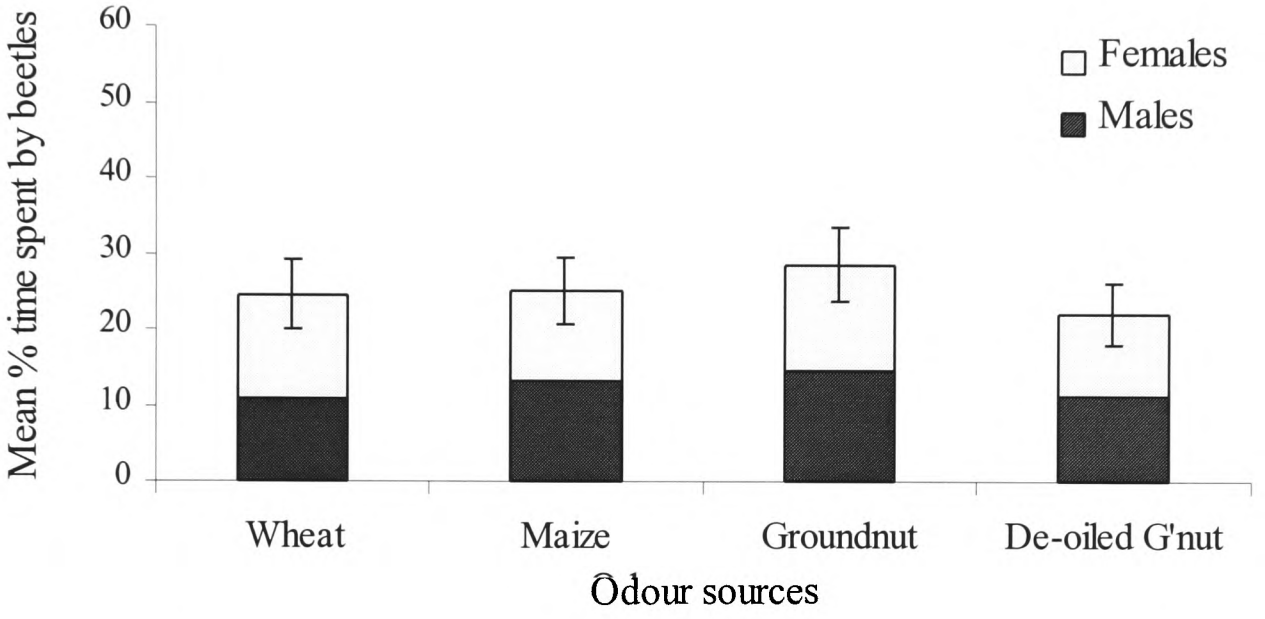


Figure 3.6: Mean percentage of the time (5 minutes) spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving volatiles from wheat, maize, groundnut or de-oiled groundnut; error bars represent the standard error for males and females combined (n=40)
NB. De-oiled G'nut = De-oiled Groundnut

A quick review of the results

The different choices of host-grain volatiles offered to the insects in different tests are presented below. Those commodities for which the beetles showed a significantly greater positive response are listed in bold face. In all experiments where comparison was made with clean air, the host-grain volatiles were significantly more attractive.

- Wheat, Maize, **Groundnut**

- Wheat, Maize, De-oiled groundnut
- Groundnut, **De-oiled groundnu**t, Groundnut oil
- Groundnut, **De-oiled groundnut**
- Wheat, De-oiled groundnut
- Maize, De-oiled groundnut
- Groundnut, De-oiled groundnut
- Wheat, Maize, Groundnut, De-oiled groundnut

3.4. DISCUSSION

Rhyzopertha dominica was reared on the two different hosts (wheat or split green gram) for approximately a year and then the beetles from those cultures were used to observe their comparative responses to volatiles from wheat and split green gram. The results showed that the beetles reared on one host did not prefer the volatiles of that particular host over the other. This suggests that feeding on a host grain may not enhance preference of *R. dominica* for that host as is the case in some other insects (Bernays, 1995; Landolt and Monica, 1995).

Rhyzopertha dominica has shown a complex pattern of behavioural responses when given the choice of different host-grain volatiles. In the initial experiment, the beetles were significantly more attracted to volatiles from groundnut, wheat or maize compared to clean air and attraction to groundnut volatiles was significantly greater than to wheat or maize volatiles. It was hypothesised that the stronger response to groundnut volatiles was possibly because of the stronger odour from groundnut due to its greater oil content. To test this hypothesis, groundnut kernels

were de-oiled and were used as a volatile source. Attraction of beetles to volatiles from de-oiled groundnut when presented in competition with wheat or maize, was reduced (17%) compared to the relative attractiveness of groundnut. This supported the hypothesis that the oil content of groundnut contributed to the observed stronger attractiveness of groundnut volatiles compared to wheat or maize. However, when beetles were given the choice between groundnut, de-oiled groundnut and groundnut oil, they showed significantly greater positive response to de-oiled groundnut than groundnut or groundnut oil. Results of the pairwise comparisons showed that the beetles were significantly more attracted to the volatiles from de-oiled groundnut than from normal groundnut. When the same sample of de-oiled groundnut was re-tested against groundnut after about three months, the difference in the response of beetles was not statistically significantly different, although the response to de-oiled groundnut volatiles was still 21% greater than that to normal groundnut volatiles. The beetles did not show greater attraction to de-oiled groundnut when compared with wheat or maize. When volatiles from wheat, maize, groundnut or de-oiled groundnut were offered at the same time, beetles did not show any significant preference, although attraction for groundnut was slightly greater. In short, groundnut volatiles are more attractive than those from wheat or maize, and de-oiled groundnut volatiles are more attractive than those from groundnut but not from wheat or maize.

These results were opposite to the hypothesis proposed at the start of the experiment, that *R. dominica* would show stronger attraction for wheat volatiles than groundnut, as wheat is the host on which it is most productive. Adults of another bostrichid, *Prostephanus truncatus,* that is also a pest of stored food, show no response to volatiles of non-host cowpea (Scholz, 1997). Many insects that are

attracted to plant volatiles prefer volatiles from host plants to non-host plants (Qiu *et al.*, 1988; Bartlet *et al.*, 1993; Gunawardena and Swarnakanthi, 1995). In some cases volatiles of a non-host plant may have a deterrent effect, and if mixed with host volatiles, can reduce the response to host-plant volatiles (Tingle and Mitchell, 1991) or even to aggregation pheromones (Byers *et al.*, 1998). Although attraction to non-host volatiles has been reported for some of the phytophagous insects such as the European grapevine moth, *Lobesia botrana* (Gabel *et al.*, 1992), it is surprising that *R. dominica* has a preference for volatiles from an unsuitable host or non-host (groundnut) over a favoured host (wheat). Hougen *et al.* (1971) reported that different cereals and oilseeds generally emit the same volatile components but in different relative amounts. Hardie *et al.* (1995) reported that two adult forms of black bean aphid (*Aphis fabae*) with different host-plant preferences could not perceive a difference between the host plants at the level of peripheral olfactory receptors. It is possible that volatiles important to the insect from wheat, maize or groundnut may consist of more or less the same components, and the beetles are responding to the same components present in the volatiles of all three host grains. Groundnut's greater oil content may have made its volatiles more attractive than wheat or maize volatiles. Attraction to grain oils has been reported for a few stored grain insects such as *Trogoderma glabrum* Everts (Nara *et al.*, 1981), *Sitophilus oryzae* (L.) (Trematerra and Girgenti, 1989) and *Tribolium castaneum* Herbst (Phillips *et al.*, 1993). This suggests that *R. dominica* cannot necessarily discriminate between a suitable and an unsuitable host or non-host on the basis of host volatiles alone.

Although the strain of *R. dominica* used in the present studies could not reproduce on groundnut, some strains of this species do actually feed and breed on groundnut.

Mukherjee and Nandi (1993) reported that *R. dominica* populations increased more quickly on groundnut (8 to 48) than on maize (8 to19) in a twelve-week period. Host-plant specificity by insects may be a result of plant-insect co-evolution. Those insect species that do not adapt to a genetic change in host-plant chemistry during evolution are not able to eat the plant as effectively as others that do adapt (Byers, 1995). In consequence, one or few species become major pests of that plant because of reduced competition between different species or in some cases even between different strains of the same species. It is therefore possible, that originally groundnut may have been a host for this strain of *R. dominica* (as they perceive it as an attractive host) but has lost the ability to feed on it in favour of wholly cereal diet. This would indicate that this strain is still going through evolving its host relationships, as it has lost the ability to feed on groundnut but still perceives it as a suitable food from its volatiles. It is also possible that the variety of groundnut used in this study was unsuitable as a host for this strain of *R. dominica*, another variety might have supported its breeding.

The stronger attraction of the beetles for volatiles from de-oiled groundnut over those from groundnut is confusing as de-oiled groundnut volatiles are not more attractive than those from wheat or maize. On the basis of present results it is difficult to suggest a reasonable hypothesis to explain this observation.

Adults of the related species *Prostephanus truncatus,* (Horn) did not fly in response to maize grains or its volatiles (Fadamiro *et al.*, 1998) but showed a weak but positive response for the volatiles from maize grains or ground maize in a walking bioassay (Scholz, 1997). The present studies have demonstrated that *R. dominica* is attracted towards host volatiles in the short-range bioassay under laboratory

conditions, but the results do not clearly demonstrate whether or not *R. dominica* can select a suitable host on the basis of host volatiles alone and tell us nothing about how the beetle might respond in flight.

Chapter: 4

Is the response of *R. dominica* to host-grain volatiles modified in the presence of aggregation pheromone?

4.1. INTRODUCTION

Studies of the behavioural response of *R. dominica* adults towards different host grain volatiles previously showed their stronger attraction to groundnut (unsuitable/non-host) volatiles than to wheat or maize (suitable host) volatiles (see Chapter 3). These findings suggested that *R. dominica* adults are not able to select a suitable host on the basis of host volatiles alone. This raises the question, if the beetles are not able to determine the suitability of a host from its volatiles then is there any other mechanism that provides information about the suitability or quality of the host before they have any physical contact with it?

Male-produced aggregation pheromones in stored-grain insect pests are generally released when the insects are feeding or food is present (Burkholder, 1970; Faustini *et al.*, 1982; Walgenbach *et al.*, 1983; Walgenbach and Burkholder, 1986; Walgenbach *et al.*, 1987; Trematerra and Girgenti, 1989; Hussain *et al.*, 1994), and the same is true for *R. dominica* (Mayhew and Philips, 1994). Therefore, the production of pheromone in these insects indicates to conspecifics the presence of an available food source.

Enhancement of attraction responses to male pheromone signals by host odours occurs in several insects (Landolt and Phillips, 1997). Greater attraction to a

mixture of host volatiles and pheromone than host volatiles or pheromone alone is known for several insect pests of crops (Dickens, 1986; 1989; Landolt *et al.*, 1994) and forest trees (Wood, 1982; Bartelt *et al.*, 1993; Petroski and Vaz, 1995). Several insect pests of stored grains such as *Trogoderma* spp. (Barak, 1989), *Sitophilus oryzae* (Trematerra and Girgenti, 1989), *Sitophilus oryzae* and *Tribolium castaneum* (Phillips *et al.*, 1993), and *Sitophilus* spp. (Wakefield, 1999), also exhibit increased responses to their aggregation pheromones when these pheromones are associated with grain odours.

Food odours have been used in pheromone-baited field traps to enhance the response of *R. dominica* adults to aggregation pheromone (Fields *et al.*, 1993; Fields and Phillips, 1994). However, the effect of combination of host volatiles and aggregation pheromone on the behavioural response of *R. dominica* adults has not been comprehensively studied. Dowdy *et al.* (1993) reported a complex pattern of responses of *R. dominica* adults to volatiles from wheat, wheat infested with *R. dominica* and synthetic pheromone sources. Volatiles from beetles in isolation from any food (it is assumed but not stated explicitly by Dowdy *et al.*, 1993) elicited only little response compared to clean air (control) from the responding beetles, although a considerable number of beetles showed a positive response to volatiles from synthetic pheromone sources. However, there was a significantly greater response to beetles plus wheat than to beetles only or wheat only when ten or more male beetles were present on the wheat. Addition of wheat volatiles to the synthetic pheromone source did not result in either a simple additive or synergistic behavioural response by the beetles. In contrast, Mayhew (1994) reported a much greater response of *R. dominica* adults to volatiles from wheat plus pheromone component D1 (synthetic) than to D1 alone.

The biological role of aggregation pheromones is not clear. Various reasons have been suggested for the occurrence of these pheromone signals such as to find suitable mates (Landolt and Phillips, 1997) or to overcome the resistance of the host (Borden, 1982). It has also been postulated that aggregation pheromone signals are exploited sex pheromone signals (Otte, 1974; Landolt and Phillips, 1997). If the basic function of male-produced pheromone signals is to find suitable mates, then one would expect a stronger response from females than males. A slightly stronger attraction of females compared with males to male-produced pheromone signals has been reported for the related species, *Prostephanus truncatus* (Horn) in field experiments (Scholz *et al.*, 1997a; Hodges *et al.*, 1998).

Three classes of experiments were undertaken. First, to test whether the pheromone signals contain any information about the quality or suitability of the food source. Second, to investigate whether the combination of host volatiles and pheromone is more attractive than pheromone alone, focusing on the natural sources of pheromone. The third series tested for any differences in responses by males and females.

For the first of these, responses of the beetles towards host-grains (wheat, maize and groundnut) were observed when male *R. dominica* were also present on them. It was hypothesised that if the pheromone signals released from males on the host grains do not contain any information about the quality of the food, then the responders would show the same order of responses as they did to host-grain volatiles alone (see Chapter 3).

For the second series of experiments, the response of *R. dominica* adults to three volatile sources, 500g maize, 5 males in 5 maize grains and 500g maize + 5 males,

was observed. It was hypothesised that 500g maize + 5 males would be more attractive than the other two odour sources, as responding beetles can not only get information about the food source through pheromone signals but also the presence of a substantial host volatile load will make the information more reliable. In initial tests odour sources were not replicated, however, there were significant differences between the odour sources. The tests were then repeated using replicated odour sources to give greater confidence in the results.

The main objectives of these studies were:

- To investigate whether or not the pheromone signals contain any information about host suitability.

- To confirm that a combination of host volatiles and aggregation pheromone is more attractive than host volatiles alone by observing beetle response to an increase in the proportion of host volatiles in the mixture.

- To determine whether there are any differences between males and females in their responses to host volatiles and host volatiles/pheromone mixtures.

4.2. MATERIALS AND METHODS

Insects

Responses of unmated beetles of known age (see section 2.2) and sex (see section 2.3) to different odour sources were observed.

Olfactometer

Details of olfactometer apparatus and experimental procedures for recording observations of behavioural responses of beetles are given in section 2.4.

Statistical analysis

See section 2.6 for statistical methods used for analysis of data.

4.2.1. Preferences of *R. dominica* adults for volatiles from different host-grains infested with male *R. dominica*

Experimental procedure

Two experiments were performed using 125g each of wheat, maize or groundnut infested with *R. dominica* males as odour sources. In the first experiment, a high ratio of aggregation pheromone to host volatiles was tested. This was prepared by placing thirty male *R. dominica* on each of the grain samples. In the second experiment a lower ratio of pheromone to grain volatiles was tested, prepared by placing only five males on each grain sample. In both cases, one-day old adult males were moved into the jars containing different host-grains, and were allowed to feed on the host-grains for seven days before using these cultures as sources of volatiles in the olfactometer experiments. In the first experiment, responses of eighty beetles (40 males and 40 females) and in the second experiment, responses of forty beetles (20 males and 20 females) to these sources of volatiles were observed. Only one sample of each odour source was used in these two experiments.

4.2.2. Nature of combined action of host volatiles and aggregation pheromone

Test on un-replicated odour sources

Three types of odour sources were used to observe responses of adult males and females of *R. dominica*. One sample of each type of odour source was used.

- Host odour alone (500g maize) –whole sound grains free of any debris were used as sources of volatiles.
- Lower ratio host volatiles/pheromone mixture (5 males in 5 maize grains) – three-day old males were moved into the maize grains (one male in one maize grain), already prepared by drilling holes in them with 1.5 mm drill. The males were allowed to feed there for four days before they were used as source of pheromone in the olfactometer tests.
- Higher ratio host volatiles/pheromone mixture (500g maize + 5 males) –five males, each in a different grain as stated above were placed on the surface of the 500g of maize grains from where they were easily retrieved at the end of the test.

It was not possible to have a natural pheromone source in complete absence of host volatiles as *R. dominica* males produce pheromone only when they are present on the food (Mayhew and Phillips, 1994). Therefore, it was necessary to have males in the grains. Thus volatiles from 5 males + 5 grains were considered a mixture of host volatiles and aggregation pheromone with a low ratio of host volatiles. Volatiles from 500g maize + 5 males were also considered as a mixture of host volatiles and aggregation pheromone but with a higher ratio of host volatiles.

In the first set of experiments (single choice), volatiles from any one of the above odour sources were delivered into the olfactometer exposure chamber at one time (from one compass point) while clean air was delivered from the other three compass points (see section 2.4). Responses of forty males and forty females were observed for each of the odour sources. In the second set of experiments (multiple choice), volatiles from all three odour sources (one from each compass point) were delivered into the exposure chamber at the same time while clean air was delivered from the fourth compass point. Responses of fifty males and fifty females were observed. The single-choice tests were undertaken to give an absolute measure of attraction while the multiple-choice tests were undertaken to demonstrate which of the three sources was preferred.

Test on odour sources replicated ten times

Volatiles of three odour sources described above were presented into the exposure chamber at the same time (one from each compass point) with clean air from the fourth compass point. Responses of five males and five females were observed, then the males used as pheromone source were interchanged between the odour sources (maize plus five males, and five males). Maize in both the odour sources (500g maize plus five males, and 500g maize) was replaced with fresh amounts. Responses of another five males and five females were observed. The whole procedure was repeated five times making ten replications of each odour source. In this way the responses of a total of 100 beetle (50 males and 50 females) were observed during the experiment.

4.3. RESULTS

4.3.1. Preferences of *R. dominica* adults for volatiles from different host-grains infested with male *R. dominica*

Preferences for volatiles from different host-grains infested with 30 males

The adults of *R. dominica* spent longer in the zone of the test arena receiving volatiles of beetle-infested wheat or maize than groundnut (Figure 4.1).

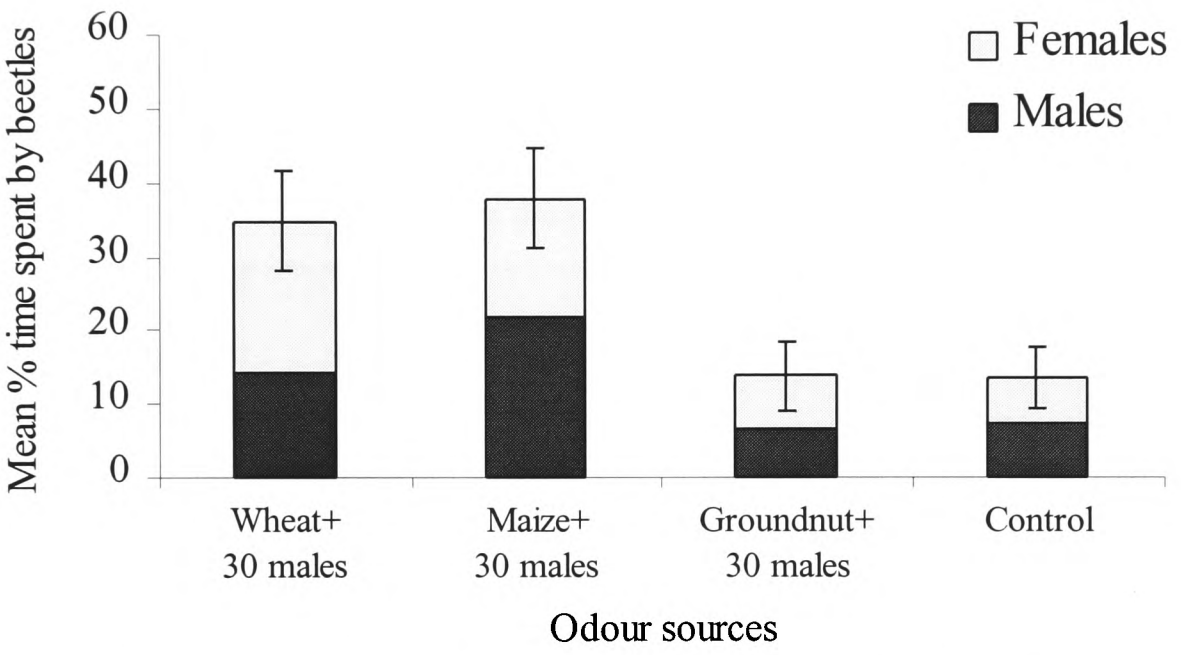


Figure 4.1: Mean percentage of the time (5 minutes) spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving volatiles from wheat, maize, groundnut, each infested by thirty *R. dominica* males, or clean air (control); error bars represent the standard error for males and females combined (n=80)

There was a significant difference between treatments for females (Chi-square=9.44, df=3, p=0.02) but not for males (Chi-square=7.40, df=3, p=0.06). The data for males and females pooled, also showed a significant difference between treatments (Chi-square=14.15, df=3, p<0.01). Analysis of the pooled data was possible as a Mann-Whitney test did not show any significant difference between males and females for beetle-infested wheat (z=-1.22, p=0.22, n=40), maize (z=-

1.06, p=0.29, n=40), groundnut (z=-0.72, p=0.47, n=40) and control (z=-0.31, p=0.75, n=40).

Pairwise comparisons between treatments (Table-4.1) showed that female response to beetle-infested wheat was significantly greater than beetle-infested groundnut but not greater than beetle-infested maize. Pairwise comparisons between treatments for the pooled data showed that responses to volatiles of beetle-infested wheat and beetle-infested maize were not significantly different from each other but both of them were significantly different from beetle-infested groundnut.

Table-4.1: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from wheat, maize, groundnut, each infested by thirty *R. dominica* males, or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Females		Pooled	
	Z	p-value	Z	p-value
Wheat *vs.* Maize	-0.88	0.38	-0.08	0.94
Wheat *vs.* Groundnut	-2.48	<0.01	-2.96	<0.01
Wheat *vs.* Control	-2.83	<0.01	-3.17	<0.01
Maize *vs.* Groundnut	-1.75	0.08	-3.14	<0.01
Maize *vs.* Control	-2.08	0.04	-3.41	<0.001
Groundnut *vs.* Control	-0.00	1.00	-0.57	0.57

Preferences for volatiles from different host-grains infested with 5 males

As in the first experiment, the adults of *R. dominica* spent longer in the zone of test arena receiving volatiles of beetle-infested wheat or beetle-infested maize than beetle-infested groundnut (Figure 4.2). As with the earlier test with 30 males, there

was a significant difference between treatments for females (Chi-square=10.63, df=3, p<0.01) but not for males (Chi-square=3.14, df=3, p=0.37). The data for males and females pooled, also showed significant difference between treatments (Chi-square=8.71, df=3, p=0.03). Analysis of the pooled data was possible as a Mann-Whitney test did not show any significant difference between males and females for beetle-infested wheat (z=-1.38, p=0.17, n=20), beetle-infested maize (z=-0.74, p=0.48, n=20), beetle-infested groundnut (z=-0.76, p=0.45, n=20) or the control (z=-0.80, p=0.46, n=20).

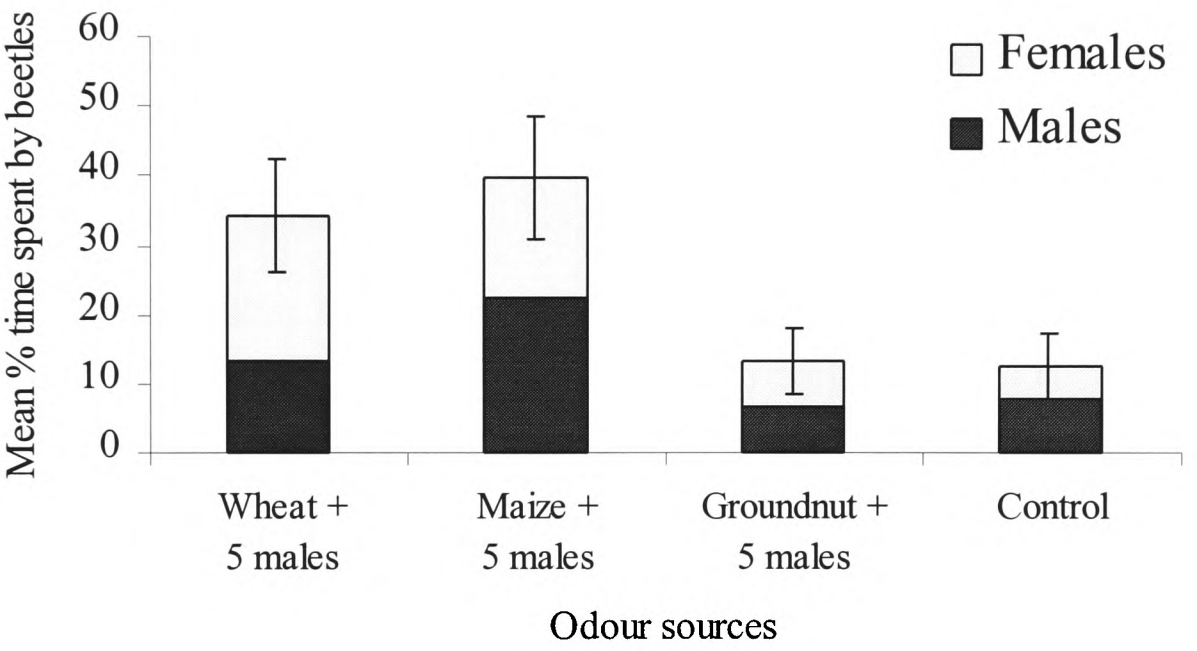


Figure 4.2: Mean percentage of the time (5 minutes) spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving volatiles from wheat, maize, groundnut, each infested by five *R. dominica* males, or clean air (control); error bars represent the standard error for males and females combined (n=40)

Subsequent pairwise comparisons between treatments (Table-4.2) also showed results similar to the previous experiment, as female response to beetle-infested wheat was significantly greater than the beetle-infested groundnut but not greater than beetle-infested maize. Pairwise comparisons between treatments for the pooled data showed that responses to volatiles of beetle-infested wheat and maize

were not significantly different from each other but responses to both of them were significantly greater than to beetle-infested groundnut.

Table-4.2: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from wheat, maize, groundnut, each infested by five *R. dominica* males, or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Females		Pooled	
	Z	p-value	Z	p-value
Wheat *vs.* Maize	-0.54	0.59	-0.36	0.72
Wheat *vs.* Groundnut	-2.44	0.02	-2.79	<0.01
Wheat *vs.* Control	-2.73	<0.01	-2.78	<0.01
Maize *vs.* Groundnut	-1.86	0.06	-2.94	<0.01
Maize *vs.* Control	-1.99	0.047	-2.86	<0.01
Groundnut *vs.* Control	-879	0.379	-0.54	0.59

4.3.2. Nature of combined action of host volatiles and aggregation pheromone

Test on un-replicated odour sources

- *Response to host volatiles alone*

Adult *R. dominica* spent more time in the zone receiving volatiles of maize than the zones receiving clean air (control zones) (Figure 4.3). There was significant heterogeneity among treatments for males (Chi-square=21.24, df=3, p<0.001) and females (Chi-square=35.07, df=3, p<0.001). Subsequent pairwise analysis (Table-4.3) of the data between different treatments for males and females showed significant difference between zone receiving volatiles from maize and zones

receiving clean air (controls). None of the controls were significantly different from each other.

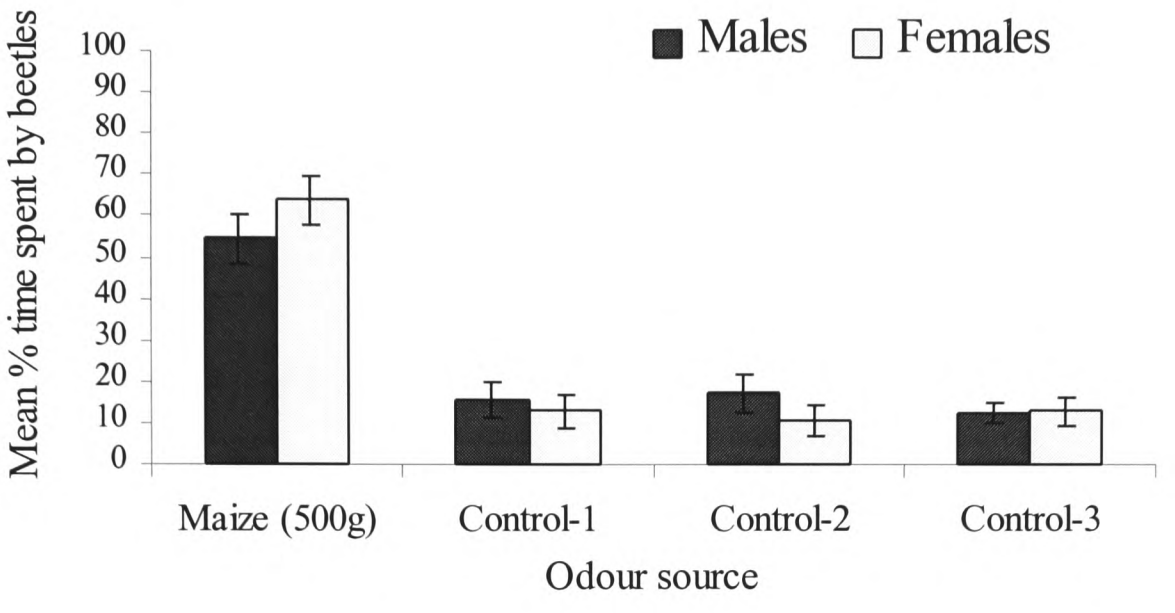


Figure 4.3: Mean percentage of the time (5 minutes) spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving maize volatiles or clean air (control); error bars represent standard error (n=40)

Is there any difference between the responses of males and females to host volatiles?

There was no significant difference between responses of males and females to maize ($z=-1.21$, $p=0.23$, $n=40$), control-1 ($z=-0.03$, $p=0.98$, $n=40$), control-2 ($z=-1.01$, $p=0.31$, $n=40$) or control-3 ($z=-0.47$, $p=0.64$, $n=40$). This result made it possible to analyse the data for males and females pooled. As expected there was a significant difference among treatments (Chi-square=55.19, df=3, $p<0.001$). Pairwise comparisons between treatments (Table- 4.3) showed that the percentage of time spent in the zone receiving maize volatiles was significantly greater than all the control zones, and that none of the control zones were significantly different from each other.

Table-4.3: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving maize volatiles or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females		Pooled	
	Z	p-value	Z	p-value	Z	p-value
Maize *vs.* Control-1	-3.62	<0.001	-4.14	<0.001	-5.48	<0.001
Maize *vs.* Control-2	-3.30	<0.001	-4.47	<0.001	-5.57	<0.001
Maize *vs.* Control-3	-4.44	<0.001	-4.49	<0.001	-6.20	<0.001
Control-1 *vs.* Control-2	-0.09	0.93	-0.79	0.427	-0.44	0.66
Control-1 *vs.* Control-3	-0.33	0.74	-0.48	0.63	-0.61	0.54
Control-2 *vs.* Control-3	-0.34	0.74	-0.70	0.48	-0.27	0.79

- *Response to the lower ratio host volatiles/pheromone mixture*

Both male and female beetles spent more time in the zone receiving volatiles from five males in five maize grains than the zones receiving clean air (control zones) (Figure 4.4). There was significant heterogeneity among treatments for males (Chi-square=49.59, df=3, $p<0.001$) and females (Chi-square=78.13, df=3, $p<0.001$).

Subsequent pairwise analysis of the data between different treatments (Table-4.4) for males and females showed significant difference between zones receiving volatiles from five males in five maize grains and zones receiving clean air (controls). None of the controls were significantly different from each other.

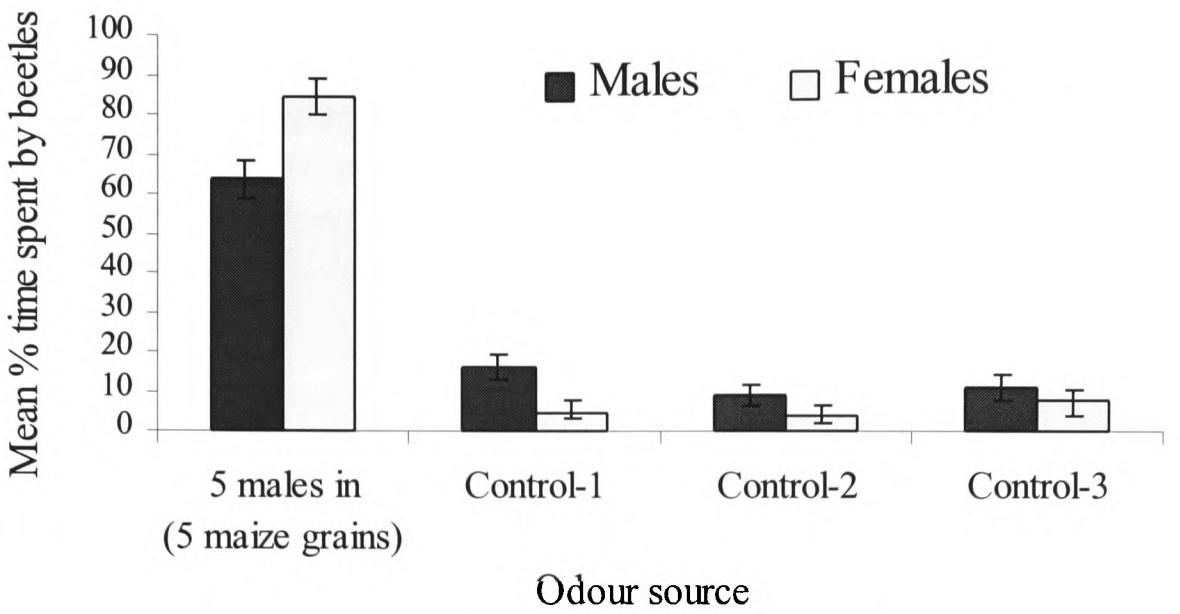


Figure 4.4: Mean percentage of time (5 minutes) spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving the lower ratio host volatiles/pheromone mixture or clean air (control); error bars represent standard error (n=40)

Is there any difference between the responses of males and females to the lower ratio host volatiles/pheromone mixture?

Female response to the lower ratio host volatiles/pheromone mixture was 24% greater than that of males. Statistical analysis confirmed this, as it showed significant difference between males and females ($z=-3.82$, $p<0.001$, $n=40$). Significantly greater response to odour source made the female response significantly smaller than that by males to control zones ($z=-3.09$, $p<0.01$, $n=40$; $z=-2.44$, $p=0.02$, $n=40$; $z=-2.25$, $p=0.03$, $n=40$).

Table-4.4: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from the lower ratio host volatiles/pheromone mixture or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females	
	Z	p-value	Z	p-value
Lower ratio mixture *vs.* Control-1	-4.60	<0.001	-5.47	<0.001
Lower ratio mixture *vs.* Control-2	-4.92	<0.001	-5.43	<0.001
Lower ratio mixture *vs.* Control-3	-4.93	<0.001	-4.80	<0.001
Control-1 *vs.* Control-2	-1.89	0.06	-0.94	0.35
Control-1 *vs.* Control-3	-1.10	0.27	-0.22	0.82
Control-2 *vs.* Control-3	-0.80	0.42	-1.04	0.30

- *Response to the higher ratio host volatiles/pheromone mixture*

As in earlier cases, adult *R. dominica* spent more time in the zone of test arena receiving volatiles than the zones receiving clean air (control zones) (Figure 4.5).

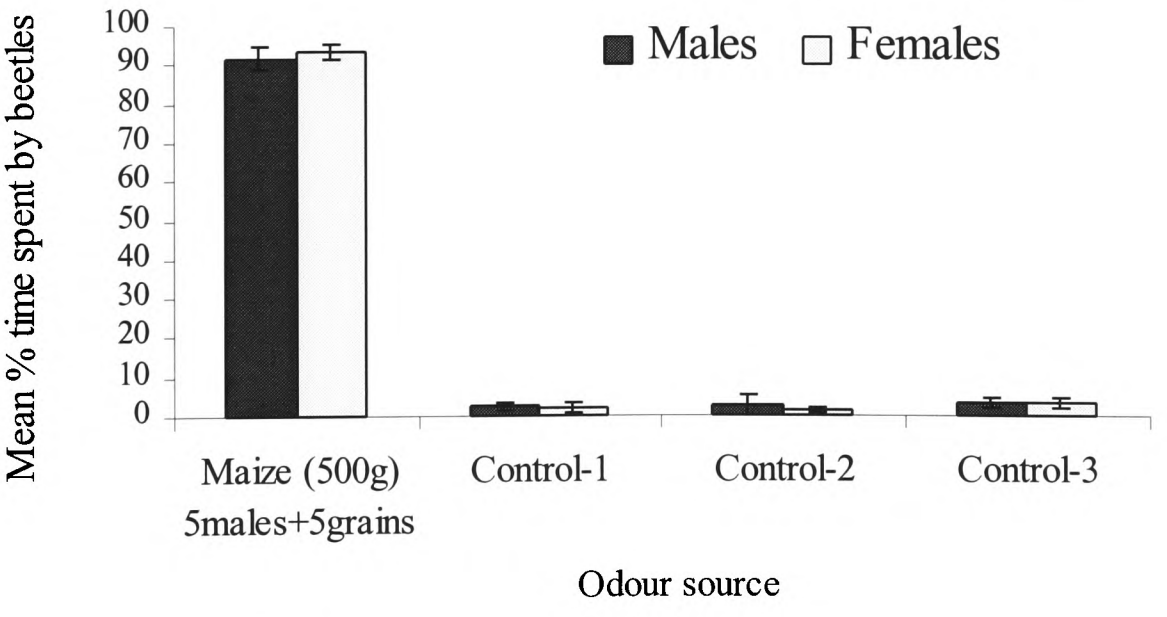


Figure 4.5: Mean percentage of time (5 minutes) spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving the higher ratio host volatiles/pheromone mixture volatiles or clean air (control); error bars represent standard error (n=40)

The response of beetles to the higher ratio mixture was stronger than that to host volatiles alone or the lower ratio mixture. There was significant heterogeneity among treatments for males (Chi-square=94.84, df=3, $p<0.001$) and females (Chi-square=102.35, df=3, $p<0.001$).

Subsequent pairwise analysis of the data between different treatments (Table-4.5) for males and females showed significant difference between zones receiving volatile mixture with a higher ratio of host volatiles to pheromone and zones receiving clean air (controls). None of the controls were significantly different from each other.

Table-4.5: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving the higher ratio host volatiles/ pheromone mixture volatiles or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females	
	Z	p-value	Z	p-value
Higher ratio mixture *vs.* Control-1	-5.60	<0.001	-5.73	<0.001
Higher ratio mixture *vs.* Control-2	-5.29	<0.001	-5.73	<0.001
Higher ratio mixture *vs.* Control-3	-5.60	<0.001	-5.73	<0.001
Control-1 *vs.* Control-2	-1.47	0.14	-0.06	0.95
Control-1 *vs.* Control-3	-0.11	0.91	-0.55	0.58
Control-2 *vs.* Control-3	-1.60	0.11	-1.26	0.21

Is there any difference between the responses of males and females to the higher ratio host volatiles/pheromone mixture?

There was no significant difference in responses between males and females for the higher ratio mixture (maize+5males) (z=-0.67, p=0.51, n=40) or control sections (z=-1.16, p=0.25, n=40; z=-0.80, p=0.42, n=40; z=-0.23, p=0.82, n=40).

- *Preference of* R. dominica *adults for host volatiles alone, lower or higher ratio host volatiles/pheromone mixtures*

Beetles spent more time in the zone receiving the higher ratio host volatiles/pheromone mixture than the zones receiving host volatiles alone or the lower ratio mixture (Figure 4.6). There was significant heterogeneity among treatments for males (Chi-square=58.15, df=3, p<0.001) and females (Chi-square=70.14, df=3, p<0.001).

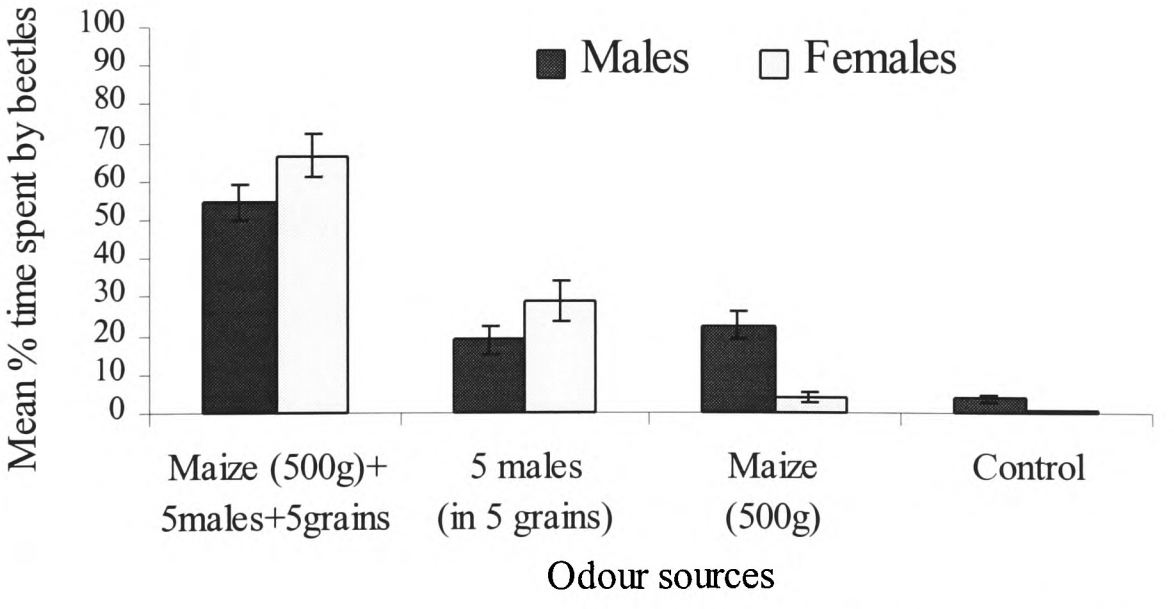


Figure 4.6: Mean percentage of time (5 minutes) spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving host volatiles alone, or the lower or higher ratio host volatiles/pheromone mixture volatiles or clean air (control); error bars represent standard error (n=40)

Subsequent pairwise analysis of the data for males showed a significant difference between all the treatment combinations except for host volatiles alone and the lower ratio mixture (Table-4.6). Whereas all the treatment combinations for females showed a significant difference.

Table-4.6: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving host volatiles alone, or the lower or higher ratio host volatiles/pheromone mixture volatiles or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females	
	Z	p-value	Z	p-value
Higher ratio mixture *vs.* Lower ratio mixture	-3.99	<0.001	-3.18	<0.001
Higher ratio mixture *vs.* Host volatiles alone	-4.03	<0.001	-5.76	<0.001
Higher ratio mixture *vs.* Control	-5.80	<0.001	-5.77	<0.001
Lower ratio mixture *vs.* Host volatiles alone	-0.75	0.45	-3.60	<0.001
Lower ratio mixture *vs.* Control	-3.86	<0.001	-4.46	<0.001
Host volatiles alone *vs.* Control	-4.44	<0.001	-2.10	0.04

Is there any difference between the responses of males and females?

Females showed significantly greater response than males to the higher ratio mixture ($z = -2.19$, $p = 0.03$, $n = 40$) while they showed a significantly lower response to host volatiles alone ($z = -5.69$, $p < 0.001$, $n = 40$). There was no significant difference between males and females to the lower ratio mixture ($z = -0.76$, $p = 0.45$, $n = 40$) although 34% more females were attracted.

Test on odour sources replicated ten times

- *Preference of* R. dominica *adults for host volatiles alone, lower or higher ratio host volatiles/pheromone mixtures*

Adult *R. dominica* spent more time in the zone receiving volatiles of the higher ratio mixture than the zone receiving host volatiles alone, the lower ratio mixture or clean air (control) (Figure 4.7). There was significant heterogeneity among treatments for males (Chi-square=56.80, df=3, $p<0.001$) and females (Chi-square=85.05, df=3, $p<0.001$).

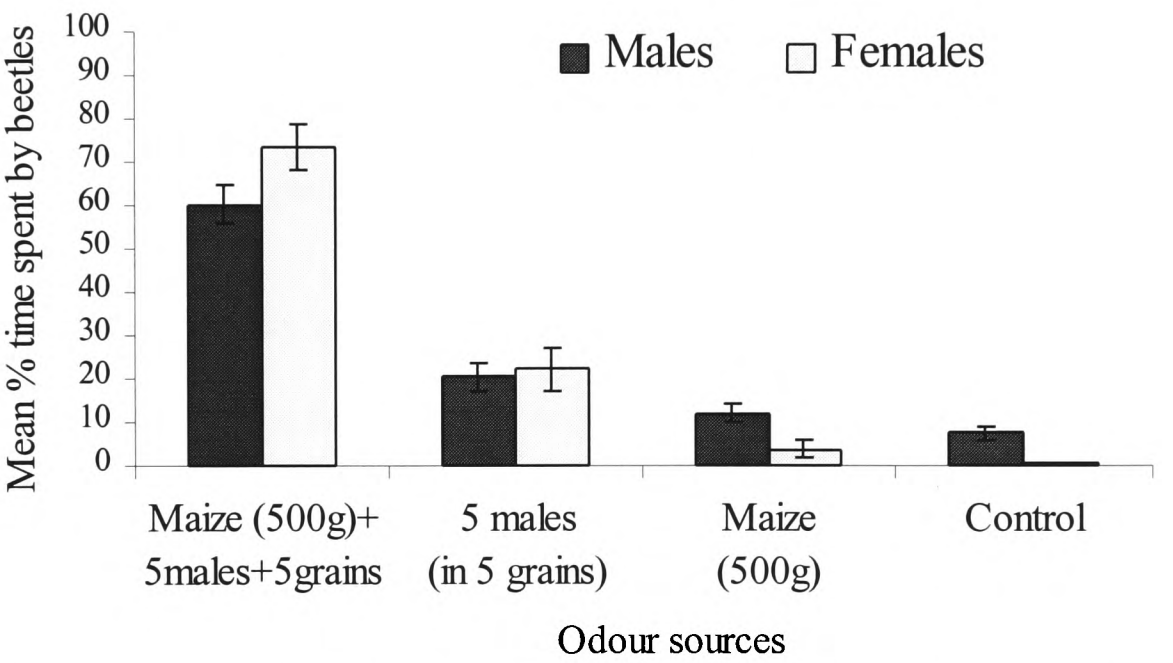


Figure 4.7: Mean percentage of time (5 minutes) spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving host volatiles alone, the lower or higher ratio host volatiles/pheromone mixtures or clean air (control); odour sources were replicated ten times; error bars represent standard error (n=50)

Subsequent pairwise analysis of the data for males, based on percentage of time spent in each zone, showed a significant difference between all the treatment combinations except between host volatiles alone and the lower ratio mixture (Table-4.7). For females the only treatment combination that was not significantly different was between host volatiles alone and clean air (control).

Table-4.7: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving host volatiles alone, the lower or higher ratio host volatiles/pheromone mixtures or clean air (control); odour sources were replicated ten times; data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females	
	Z	p-value	Z	p-value
Higher ratio mixture *vs.* Lower ratio mixture	-4.27	<0.001	-3.96	<0.001
Higher ratio mixture *vs.* Host volatiles alone	-5.42	<0.001	-5.54	<0.001
Higher ratio mixture *vs.* Control	-5.65	<0.001	-5.83	<0.001
Lower ratio mixture *vs.* Host volatiles alone	-1.91	0.06	-3.58	<0.001
Lower ratio mixture *vs.* Control	-3.57	<0.001	-4.36	<0.001
Host volatiles alone *vs.* Control	-2.18	0.03	-1.86	<0.06

Is there any difference between the responses of males and females?

Females showed significantly greater positive response than males to volatile mixture with a higher ratio of host volatiles to pheromone ($z=-2.46$, $p=0.01$, $n=40$) while males showed significantly greater positive response to host volatiles alone ($z=-4.84$, $p=0.001$, $n=40$). There was no significance difference between males and females to volatile mixture with a lower ratio of host volatiles to pheromone although 8% more females were attracted to the mixture.

4.4. DISCUSSION

4.4.1. Preferences of *R. dominica* adults for volatiles from different host-grains infested with male *R. dominica*

In both the experiments, when 125gm of host grain was infested with 30 males or with 5 males, the responding beetles showed stronger positive response for the

volatiles of the beetle-infested wheat or beetle-infested maize than beetle-infested groundnut. The beetles (males + females) showed 21% and 24% greater response to wheat and maize, respectively, than groundnut when commodities were infested with thirty *R. dominica* males. When commodities were infested with five *R. dominica* males, the beetles showed 21% and 26% greater response to wheat and maize, respectively, than groundnut. The similar level of response in both the cases when commodities were infested with either 5 or 30 males, suggests that beetle's response to pheromone is not enhanced with the increasing quantity of pheromone after it reaches to a certain threshold level. On the other hand it is also possible that similar quantities of pheromone were being released by groups of 5 or 30 males and hence similar response. This has been investigated in Chapter 7. This also indicates that in stores a few *R. dominica* males infesting a commodity are potentially capable of causing a serious infestation by attracting other individuals.

It was hypothesised that a combination of groundnut volatiles and aggregation pheromone (beetle-infested groundnut) would get a stronger positive response than a combination of wheat or maize volatiles and aggregation pheromone, as groundnut volatiles on their own are more attractive than wheat or maize volatiles (see Chapter 3). However, the beetles preferred a combination of host wheat or maize volatiles and aggregation pheromone over the non-host groundnut volatiles/pheromone mixture. This indicates that, either pheromone signals are not produced by the beetles on non-host groundnut or they are less attractive to responders than that produced on hosts wheat or maize. It has been suggested that chemical constituent of food-plants can affect, in part, the ability of certain insects to produce sex pheromone (Hendry *et al.*, 1980). The present studies indicate that the pheromone signals produced on a host convey some information about the

suitability of the food source to the receivers. It further suggests that, in the combination of host volatiles and aggregation pheromone, the aggregation pheromone is a stronger stimulus for responders than the host volatiles. Possible reasons for the lower attractiveness of pheromone produced on non-host groundnut may be that either the quantity or/and ratio of the pheromone components released is less favourable. Whether *R. dominica* males produce any pheromone signals on groundnut, and if they do, whether these signals really differ from those produced by beetles on wheat or maize, is addressed in Chapter 6.

4.4.2. Nature of combined action of host volatiles and aggregation pheromone

When the host volatiles alone, lower or higher ratio host volatiles/pheromone mixtures were presented in the olfactometer exposure chamber singly (single-choice tests), the beetles showed a stronger positive response to the higher ratio mixture (92% males & 93% females) than to lower ratio mixture (64% males & 84% females) or host volatiles alone (55% males & 64% females).

In the multiple-choice tests, when volatiles from all three odour sources were presented in the exposure chamber at the same time, both male and female beetles showed a significantly stronger preference to the higher ratio mixture than the other two odour sources or control. Male response to the higher ratio mixture was greater than the lower ratio mixture or host volatiles alone by 48% and 42%, respectively. For females the same comparisons were 39% and 89%, respectively. It can be argued that basing results on only a single sample of odour sources may give an unreliable indication of the results. These doubts were effectively removed by the results of subsequent tests in which multiple samples of odour sources were used and similar results obtained. In this case, male response to higher ratio

mixture was 49% and 67% more than to the lower ratio mixture or host volatiles alone, respectively. Females showed even a stronger response to the higher ratio mixture, it was greater than the lower ratio mixture or host volatiles alone, by 53% and 91%, respectively. A further feature that gives confidence in these results is the design of the experiment. The groups of males used as source of pheromone in one treatment (e.g. the 5 males in 5 grains used for the lower ratio mixture) were also used as the source of pheromone in the other treatment (500g maize + 5 males in 5 grains). Therefore, it was most likely that the observed difference in the response is actually due to difference in the ratio of pheromone to host volatiles rather than difference in pheromone outputs between males.

These findings confirm the results of Dowdy *et al.* (1993) which showed that the response to wheat plus beetles was significantly greater than that to wheat only or beetles only. These results are not, however, comparable with those of Mayhew (1994) as he used a synthetic pheromone source. In the present studies (Figure 4.4) the response to 5 males in 5 grains (low ratio mixture) was significantly greater than that to control (clean air). Dowdy *et al.* (1993) found no significant difference in response between beetles alone compared to control (clean air). The explanation for this appears to be that Dowdy *et al.* (1993) did not provide food for the beetles at the time they were being used as sources of pheromone, thus beetles were probably producing little or no pheromone. This shows, even though *R. dominica* males may continue to release pheromone a few hours after food has been removed (Mayhew and Phillips, 1994), food should be provided to the beetles at the time they are being used as source of pheromone in bioassay experiments to make sure that they are producing pheromone.

The much greater attraction of *R. dominica* to the higher ratio host volatiles/pheromone mixture observed in the present studies could result from synergy between aggregation pheromone and host volatiles. However, more studies will be needed to confirm whether the combined action of host volatiles and aggregation in *R. dominica* is truly synergistic (i.e. in which the response to the combination is greater than the combined responses to the individual components, Phillips *et al.*, 1993).

Difference in male and female-response to host volatiles or aggregation pheromone

When the three sources of host volatiles and aggregation pheromone were tested singly, the females showed significantly greater response to the lower ratio mixture than did the males. Although there was no significant difference between responses of males and females to host volatiles alone or the higher ratio mixture, female response was greater than that of males in both the cases. When volatiles from all three sources were delivered into the chamber at the same time, females showed significantly greater response to the higher ratio mixture. However, responses of the sexes to the lower ratio mixture were not significantly different, even though females showed a greater response than males.

The results suggest that females are more sensitive than males to the host volatiles alone or a low ratio aggregation pheromone/host volatile mixture when either of these is presented alone (Figure 4.3 & 4.4). When host volatiles are presented at a higher ratio to aggregation pheromone then both males and females showed strong positive response to the source of volatiles (Figure 4.5). If beetles were given the choice to select between host volatiles alone or lower or higher ratio mixtures, then

females hardly spent any time in the zone receiving host volatiles alone (Figure 4.6 & 4.7). In contrast, males spent relatively more time in this zone (on average 18%), which shows that in the presence of aggregation pheromone, host volatiles are relatively less attractive for females and their response may be largely mediated by aggregation pheromone. But for the males, host volatiles appear to be of relatively greater importance compared to the lure of aggregation pheromone. The reason for this might be that males are responsible to find a food source as only they have the ability to inform other individuals about the availability of a food source through their pheromone signals. While females locate the food source by following the signals produced by males.

Chapter:5

Pheromone signalling by *R. dominica* males:

Individual variation and association with feeding/boring activity

5.1. INTRODUCTION

The study of individual variation in pheromone production in insects has gained importance in the last two decades. The fact that response to individual signallers from conspecifics varies, indicates the biological significance of the variation in pheromone signals among individuals, in the context of population dynamics and sexual selection (Svensson, 1996; Svensson *et al.*, 1997). The study of pheromone signals on an individual level makes it possible to determine the characteristic(s) of a pheromone signal that are a possible cause of an observed response. For example, the capacity of a female to attract males through her sex pheromone signals might determine the chances of her mating success and/or quality of mates. Sex pheromones produced by females vary in proportions of pheromone components in the blend among individuals, and that may be a cause of variable response from males. Studies using synthetic pheromones have shown that some ratios of female sex pheromone components are more attractive to males than are others (Kou and Chow, 1991; Leal *et al.*, 1993; Daterman *et al.*, 1995).

In many studies on pheromone production, the emphasis has been to investigate the effects of factors such as age, mating status, host plant, time of day and diurnal rhythms etc. (Mazomenos, 1984; Ono *et al.*, 1990; Kamimura and Tatsuki, 1993;

Delisle and Royer, 1994; Mayhew and Phillips, 1994; Lextrait *et al.*, 1995) on pheromone production by individuals or differences between the mean level of production of populations (Ono *et al.*, 1990; LaForest *et al.*, 1997). It has been suggested that for a few insect species, different strains can be distinguished on the basis of variation in composition of pheromone components in the blend (Buechi *et al.*, 1982; Aldrich *et al.*, 1987; Aldrich *et al.*, 1989; Aldrich *et al.*, 1993; Zhu *et al.*, 1996). The validity of identification of strains on the basis of variation in ratio of pheromone components has, however, been questioned (Ryan *et al.*, 1995), but continued studies may provide sufficient evidence to solve the problem of differentiating between morphologically indistinct strains on the basis of variation in pheromone blends (Hsu, 1984). However, not many studies have investigated the variation among individuals of the same population present under similar conditions. This has been mainly because of the difficulties in collection of pheromone from individual insects and its accurate chemical analysis. Now with the availability of more sophisticated techniques and equipment it has become possible to quantify pheromone production from single insects.

It has been demonstrated that pheromone production in *R. dominica* males is dependent on feeding and food volatiles alone do not trigger pheromone production (Mayhew and Phillips, 1994). However, it is not clear whether feeding simply triggers pheromone production or whether the rate of feeding is directly related to the quantity of pheromone produced. The different aspects of the relationship between feeding and pheromone production have been studied for a few other insects. Tillman *et al.* (1998) in radiotracer studies elucidated the relationship between feeding, juvenile hormone production and aggregation pheromone

biosynthesis in the bark beetle, *Ips pini*. They suggested that feeding on host phloem induce juvenile hormone biosynthesis that stimulates *de novo* pheromone biosynthesis in *Ips pini*. Pierce *et al.* (1986) reported that production of aggregation pheromones by males of *Oryzaephilus surinamensis* (L.) and *O. mercator* (Fauvel), *Cryptolestes ferrugineus* (Stephens) and *Tribolium castaneum* (Herbst) was enhanced by feeding on methoprene-treated oats. The latter is a juvenile hormone mimic; this suggests juvenile hormone might be implicated in pheromone production.

In the present studies, pheromone production from single *R. dominica* males of the same age and under similar conditions was quantified to investigate;

- the variation among individuals in the absolute quantities and ratio of the pheromone components produced, and
- the relationship between rate of feeding activity and the rate of pheromone production.

Later in the thesis, the influence of various factors on pheromone signalling by males is investigated. The effect of these factors on pheromone signalling may be mediated through their influence on the feeding activity. Direct measure of feeding activity is difficult so in the present studies, the quantity of the dust produced due to feeding/boring of male beetles was used as a measure of feeding/boring activity. The weights of grains, and beetles at different steps of the experiment were also recorded to investigate whether any changes in body weight of beetles were detectable.

5.2. MATERIALS AND METHODS

Insects

Rhyzopertha dominica adults were removed from culture within twenty hours after emergence, sexed (see section 2.3) and kept singly on kibbled wheat grains for three days. Then the males were moved into maize grains (one male per grain), these were already prepared by drilling approximately 4 mm deep holes in them with a 1.5 mm drill. The males were allowed to feed in the grains for four days before making pheromone entrainments.

Pheromone entrainment

Details of the equipment used in pheromone entrainments and their arrangement is presented in section 2.5.

Experimental procedure

Single *R. dominica* males were weighed and moved into individual, pre-weighed maize grains that were approximately of similar size and shape (see Figure 5.1 for illustration of experimental procedure). A Mettler AE160 type balance ($\pm$ 0.1mg) was used to weigh both grains and beetles. The grains used as control (with drilled holes like treatments but without beetles) were also weighed. The grains with beetles (treatment) and without beetles (control) were left in the CTH room running at 27°C temperature and 60% relative humidity for three days. On the fourth day, the grains with beetles (including dust produced) and without beetles (control) were weighed once again, and moved into the entrainment chambers. Ten entrainments

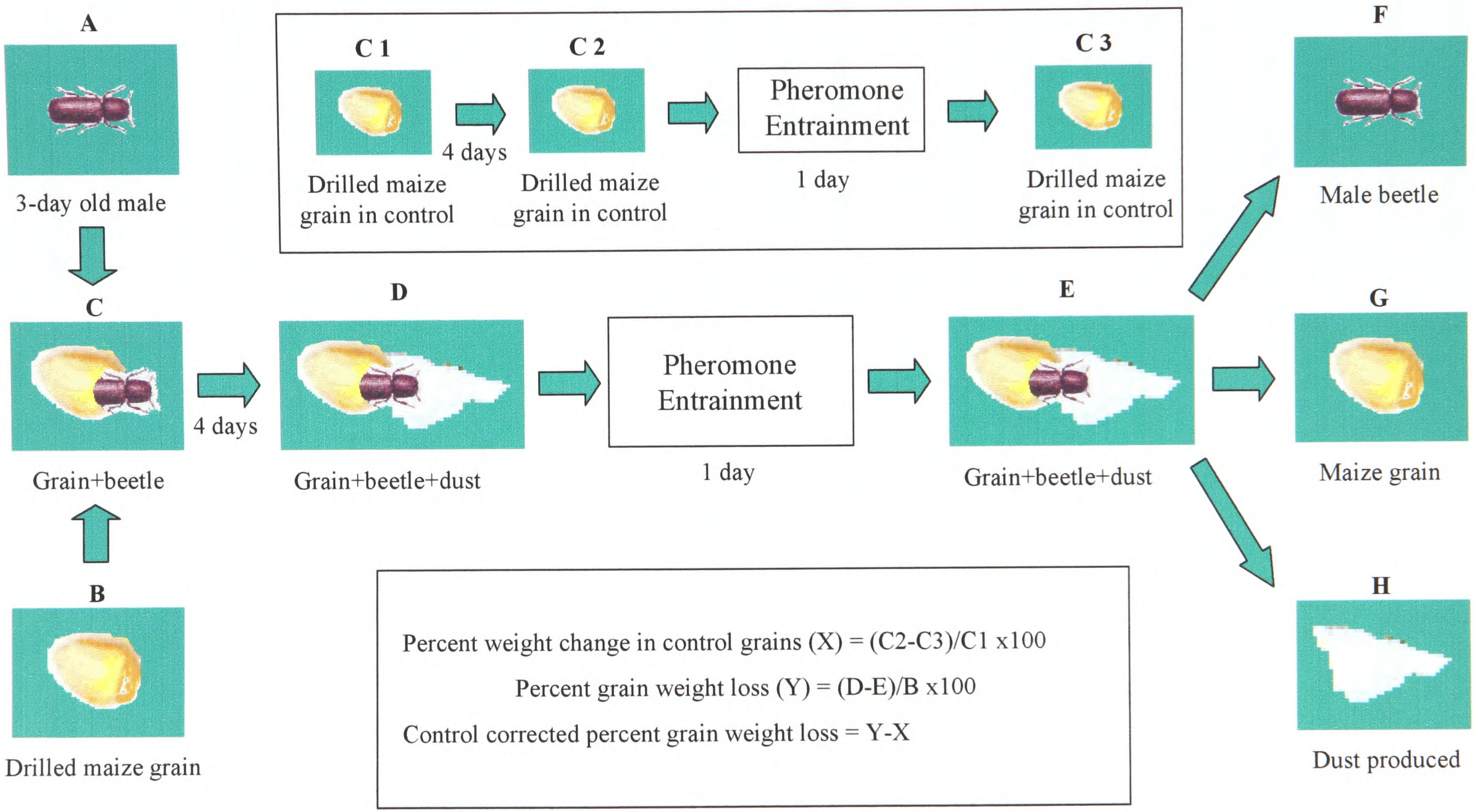


Figure 5.1: Experimental procedure illustrating how various measures were taken, and parameters of feeding/boring activity were calculated; weight was recorded on all the steps shown in the diagram and letters indicate the weight recorded

were made at a time including eight replications of treatment and two of control. This whole procedure was repeated thrice making a total of twenty-four replicates of treatment and six replicates of control. After the entrainments were made the weights of grains with beetles (including dust produced) were recorded. Beetles were then removed from the grains and weights of beetles and grains were recorded separately. Dust produced by beetles while boring into the grains was also weighed. The grains in the treatment (beetle infested) and without beetles (control) were weighed just before moving them into the pheromone entrainment chambers. Ten entrainments were made at a time including eight replications of treatment and two of control. This whole procedure was repeated thrice making a total of twenty-four replicates of treatment and six replicates of control. After the entrainments were made the weights of grains with beetles (including dust produced) were recorded. Beetles were then removed from the grains and weights of beetles and grains were recorded separately. Dust produced by beetles while boring into the grains was also weighed. When the grains in the treatment (beetle infested) and without beetles (control) were weighed just before moving them into the pheromone entrainment chambers, they showed an increase in weight. That was possibly because of increased moisture content of the grains due to high humidity in the test room. To obtain the loss in weight of grains during entrainments, the weight of grains recorded after the entrainments was subtracted from the weight of grains recorded just before they were moved into the entrainment chambers. However, control grains also lost weight after entrainment (possibly because of the loss of moisture content due to airflow), this percentage loss in the weight had to be deducted from the percentage loss in weight of treatment grains to obtain actual

loss that occurred due to feeding of beetles. These control-corrected values were used in the further analysis.

Pheromone output by *R. dominica* has at least four important characteristics, quantity of Dominicalure-1 (D1), quantity of Dominicaure-2 (D2), total quantity (D1+D2) and ratio of both the components. An attempt was made to correlate each of these pheromone characteristics with feeding/boring activity.

Statistical analysis

The Coefficient of Variation (CV = Standard deviation/Mean x 100) was calculated as a measure of amount of variation in pheromone signals among males.

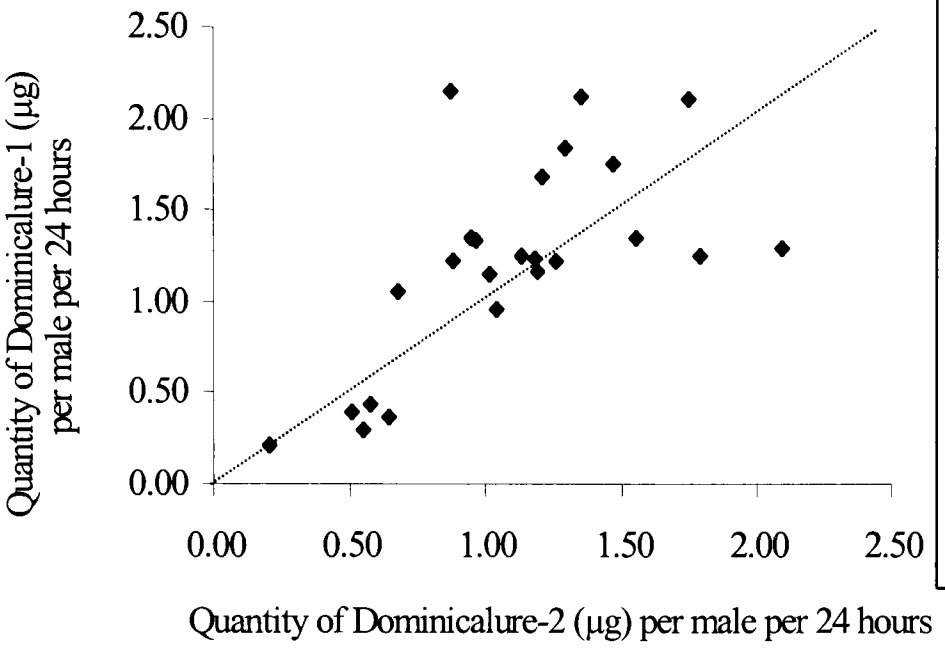
Spearman's rank test was used to correlate the data for weight of dust produced, grain weight loss, and beetle weight to different characteristics of the pheromone output. Wilcoxon Signed Ranks Test was used to compare change in the weight of beetles before and after pheromone entrainment.

5.3. RESULTS

5.3.1. Individual variation in pheromone signalling of *R. dominica* males

R. dominica males showed a considerable variation among individuals for the total quantities of the pheromone and both of its components (Figure 5.2). The range of values obtained is given in Table-5.1. In Figure 5.2, more points are towards the centre of the graph for both of the pheromone components, this shows more beetles produced quantities of pheromone that are near the mean, and fewer beetles produced much greater or much smaller quantities of pheromone. Slightly more

points being present above the 'reference line' in Figure 5.2 shows that relatively more beetles produced pheromone blend with a greater percentage of D1.



*The diagonal line shown here is just a 'reference line' for the proportion of D1 in the pheromone blend, and does not have any statistical significance. All the points above this line show that the quantity of D1 in the pheromone blend is more than 50% and below this line less than 50%.

Figure 5.2: Variation in the quantity and the ratio of the two pheromone components, Dominicalure-1 and Dominicalure-2 produced by individual *R. dominica* males

The Coefficient of Variation (CV) showed that the amount of variation in the quantity of D1, D2 and D1+D2 was similar (Table-5.1). However, the amount of variation was much smaller for the percentage of D1 in the pheromone blend than absolute quantities of the pheromone.

Table-5.1: Minimum and maximum quantities of Dominicalure-1 (D1), Dominicalure-2 (D2), total quantity of pheromone (D1+D2), and percentage of the D1 in the pheromone blend produced by single *R. dominica* males in twenty four hours feeding in maize grains; mean values and Coefficient of Variation (CV) for these pheromone characteristics are also presented (n = 24)

Pheromone characteristic	Minimum value	Maximum value	Mean	CV
D1 (µg)	0.21	2.15	1.22	46.65
D2 (µg)	0.20	2.10	1.09	41.40
D1+D2 (µg)	0.41	3.86	2.30	40.37
%D1	35.13	71.28	51.37	17.19

5.3.2. Association between the pheromone signalling of *R. dominica* and its feeding/boring activity

Relationship between quantity of dust produced due to beetle's feeding/boring activity and pheromone output

The mean quantity of dust produced per male per day, due to feeding/boring of *R. dominica* males was 1.81mg. The quantity of dust produced was variable among individuals ranging from 0.29mg to 3.63mg per male per day. Analysis of the data showed a moderately strong but statistically significant positive correlation (Figure 5.3) between quantity of dust produced and quantity of D1 ($r=0.51$, $p<0.01$, $n=24$), quantity of D2 ($r=0.52$, $p<0.01$, $n=24$) and total quantity of pheromone ($r=0.57$, $p<0.01$, $n=24$). However, there was apparently no correlation between quantity of dust and percentage of D1 in the pheromone blend produced by beetles ($r = 0.14$, $p = 0.52$, $n = 24$).

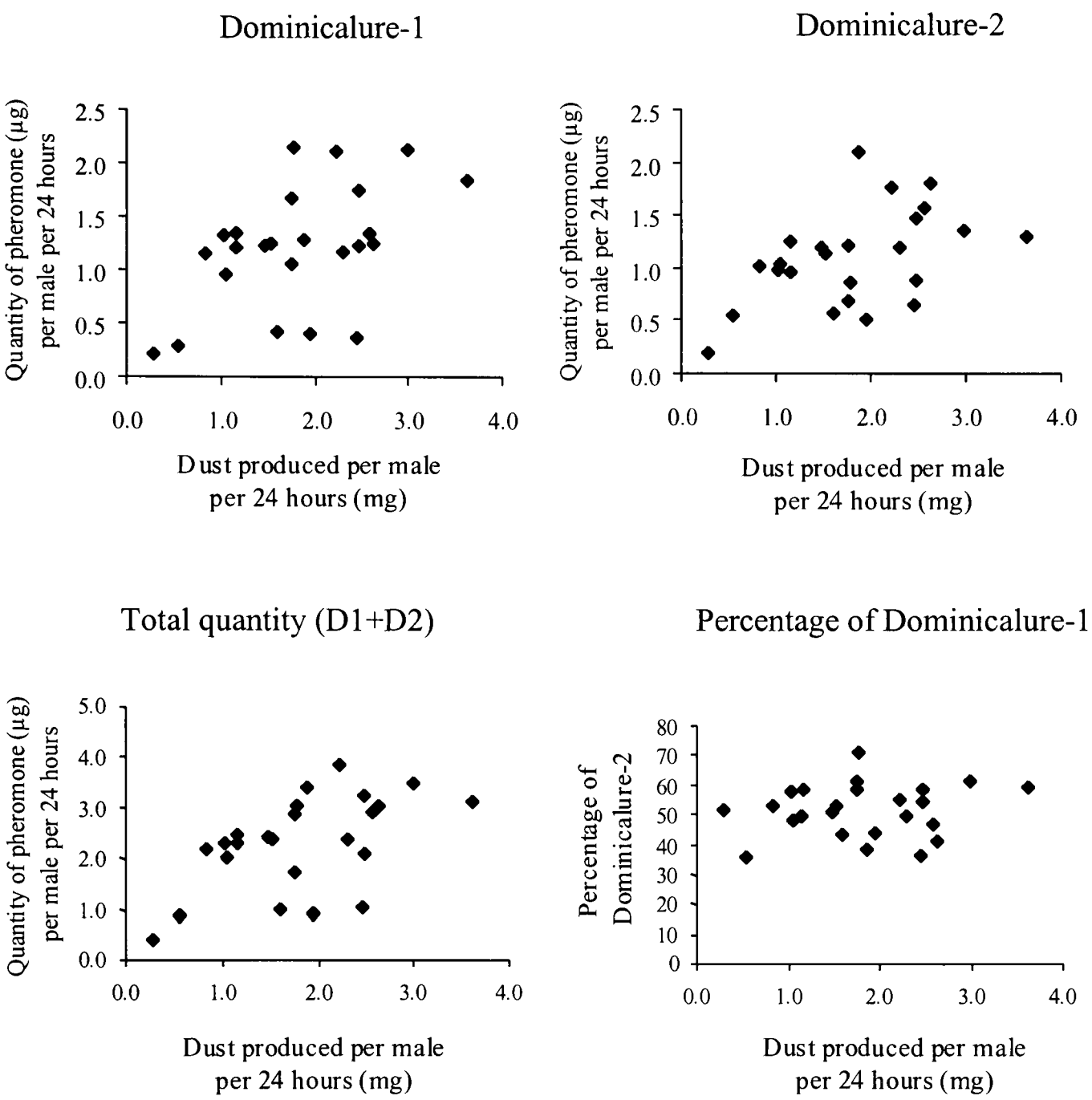


Figure 5.3: Correlation between quantity of dust produced and pheromone characteristics: quantity of pheromone component Dominicalure-1 (D1), Dominicalure-2 (D2), total quantity (D1+D2), and percentage of the D1 in the pheromone blend

Relationship between grain weight loss and pheromone output

The average weight of a maize grain was 406mg. In most of the cases the weight of the grains was reduced due to the feeding of *R. dominica* males. The mean weight of grain lost over 24 hours (control corrected) due to feeding of the beetles was 0.34% of the original weight of the grains. The maximum reduction in weight was 0.71%, however in one case no reduction in weight was observed. Statistical

analysis of the data did not show any apparent correlation (Figure 5.4) between grain weight loss and different pheromone characteristics, quantity of D1 (r=0.02, p=0.93, n=24), quantity of D2(r=-0.13, p=0.53, n=24), total quantity of pheromone (r=-0.09, p=0.69, n=24) and percentage of D1 in the pheromone blend (r=-0.12, p=0.56, n=24).

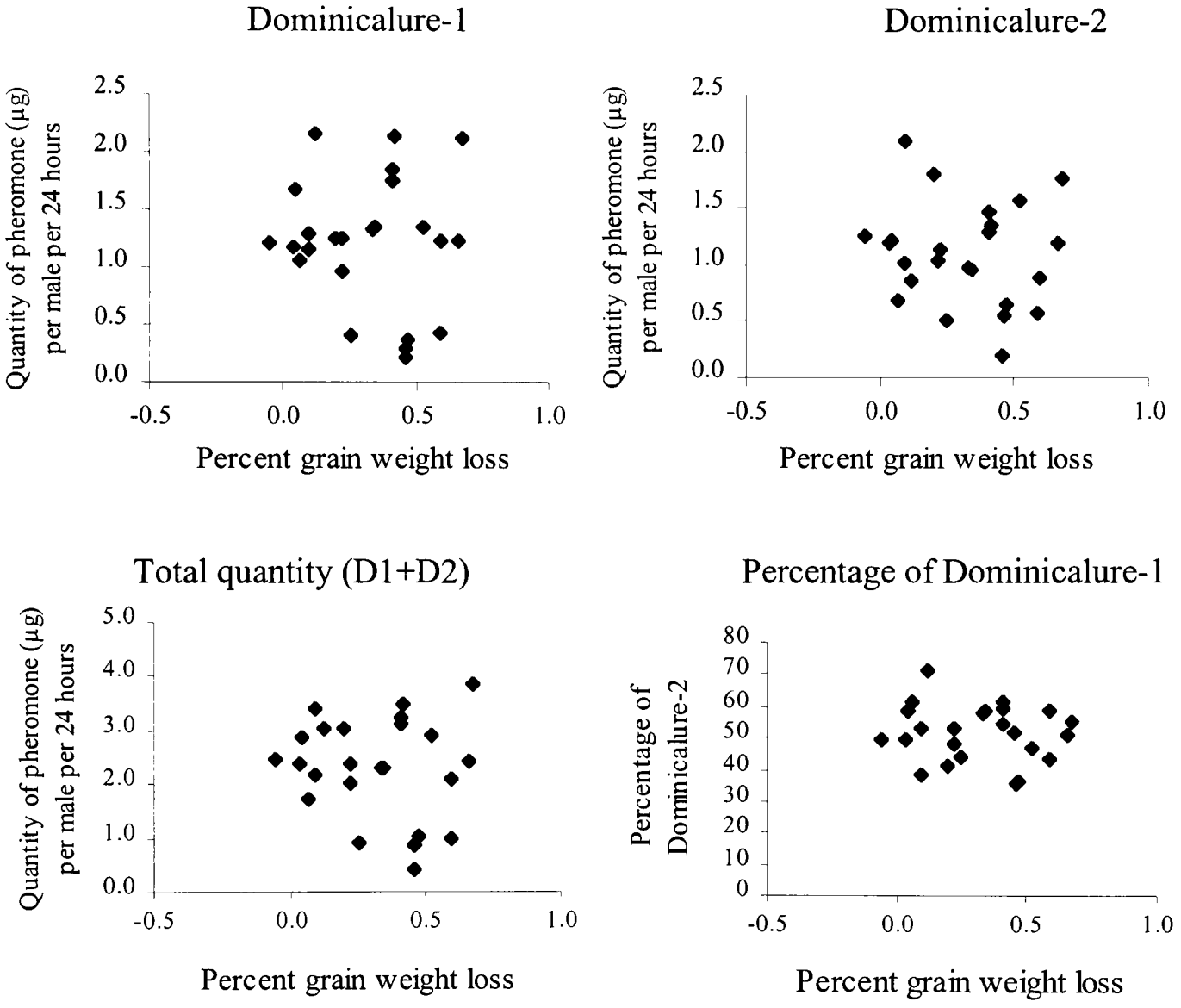


Figure 5.4: Correlation between percent grain weight loss and pheromone characteristics: quantity of pheromone component Dominicalure-1 (D1), Dominicalure-2 (D2), total quantity (D1+D2) and percentage of the D1 in the pheromone blend

5.3.3. Association between the body weight of *R. dominica* and its pheromone output or rate of feeding/boring

Relationship between beetle body weight and pheromone output

The weight of the beetles recorded at the time they were moved into the maize grains ranged from 0.63mg to 1.18mg per beetle. Statistical analysis of the data showed a moderately strong positive correlation (Figure 5.5) between weight of the beetles and quantity of D1 ($r=0.44$, $p=0.03$, $n=24$) and total quantity of the pheromone ($r=0.49$, $p=0.02$, $n=24$). However there was no apparent correlation between weight of the beetles and quantity of D2 ($r=0.37$, $p=0.08$, $n=24$) and percentage of the D1 in the blend ($r=0.02$, $p=0.93$, $n=24$). The weight of the beetles recorded after the pheromone entrainment ranged from 0.70mg to 1.30mg per beetle. As in the earlier cases there was a positive correlation between weight of the beetles and quantity of D1 ($r=0.41$, $p=0.045$, $n=24$), and total quantity of pheromone ($r=0.41$, $p=0.045$, $n=24$). While no significant correlation was found between beetle weight and D2 quantity ($r=0.24$, $p=0.26$, $n=24$), or percentage of D1 ($r=0.40$, $p=0.06$, $n=24$).

Relationship between beetle body weight and quantity of dust produced or grain weight loss

The weight of the dust produced by the beetles showed a positive and statistically significant correlation with the weight of the beetles recorded before pheromone entrainment ($r=0.58$, $p=0.003$, $n=24$) (Figure 5.6a). However, the weight of the beetles did not correlate with grain weight loss ($r=0.38$, $p=0.07$, $n=24$) (Figure 5.6b).

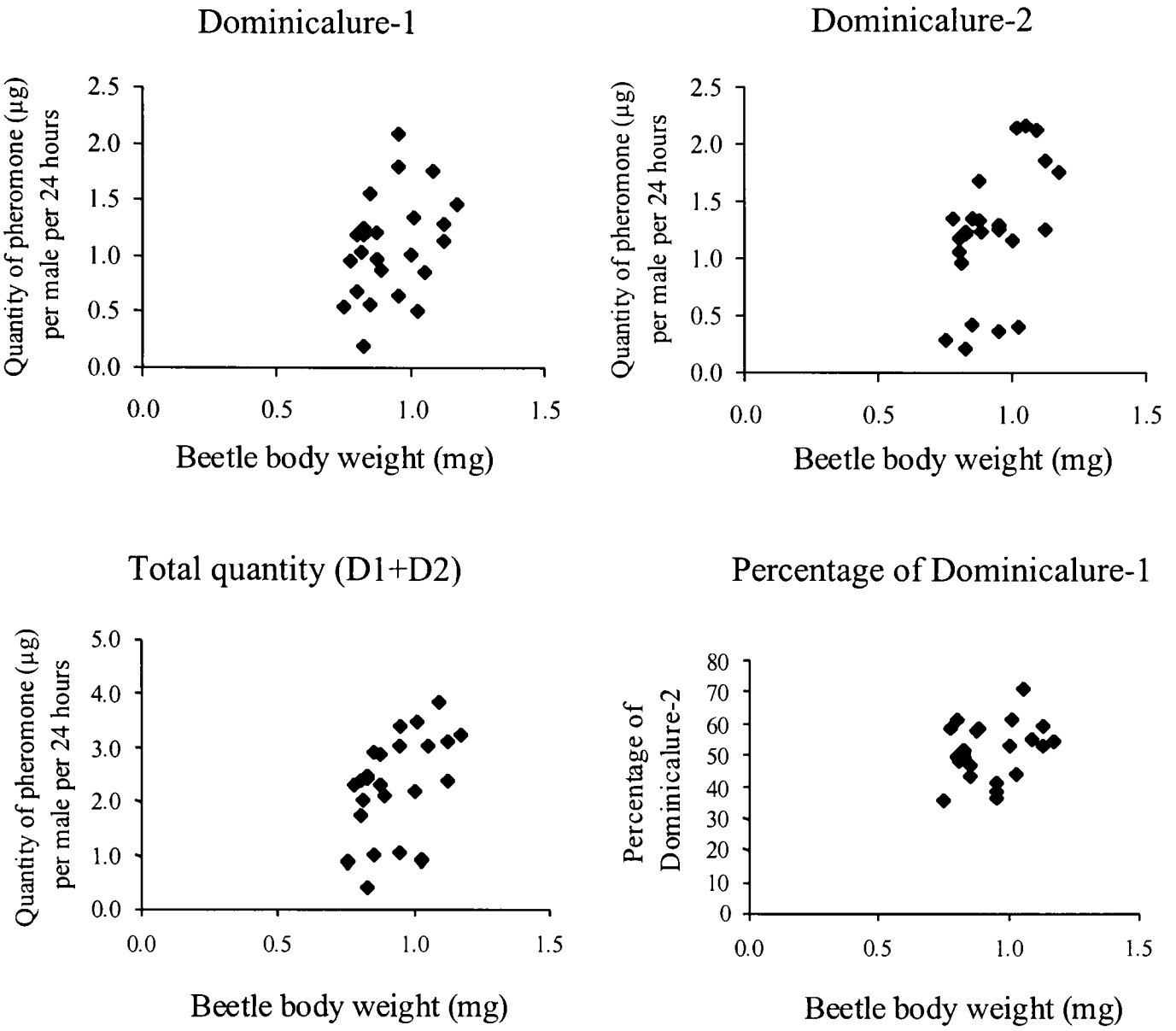


Figure 5.5: Correlation between beetle weight and pheromone characteristics, quantity of pheromone component Dominicalure-1 (D1), Dominicalure-2 (D2), total quantity (D1+D2) and percentage of the D1 in the pheromone blend

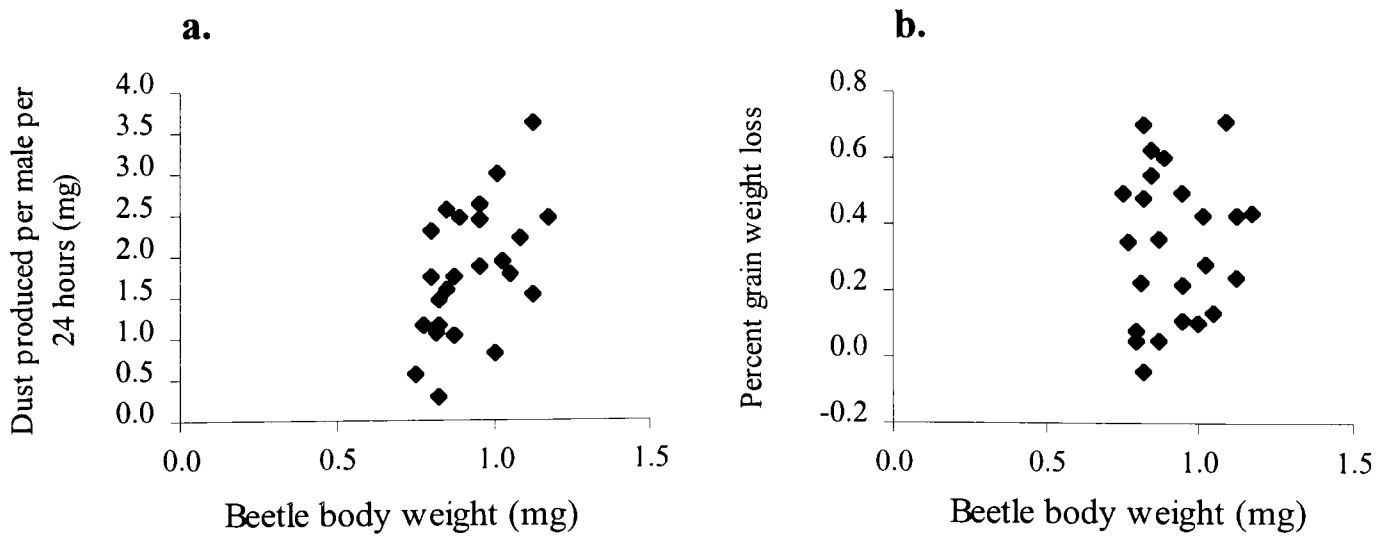


Figure 5.6: Correlation between (a) beetle weight and weight of the dust produced per male per day, and (b) beetle weight and percent grain weight loss

5.4. DISCUSSION

5.4.1. Individual variation in pheromone signalling of *R. dominica* males

In these studies *R. dominica* males of the same age present under similar situations have shown considerable individual variation in the rate of production of their aggregation pheromone. There was approximately a ten-fold difference between the minimum (D1 = 0.21µg; D2 = 0.20µg) and maximum (D1 = 2.15µg; D2 = 2.10µg) quantities of the pheromone components produced by individual beetles. Mayhew and Phillips (1994) also reported a considerable variation in the quantities of the pheromone produced by *R. dominica* males over a thirty–day period. However, their results are not strictly comparable with the results of the present studies as the variation reported by them might have resulted from difference in the age of the beetles. Schlyter and Birgersson (1989) in their review on individual variation in bark beetle and moth pheromones have presented a number of examples of cases where large individual variation in different species was observed. Birgersson *et al.* (1988) reported that total amounts and proportions of components of the aggregation pheromone of bark beetle, *Ips typographus* (L.), varied considerably among individuals excised from attacks on standing trees. Byers (1989) discussed the individual variation among bark beetles in biosynthesis, release, and response to pheromone.

The amount of variation (CV) was greater for the absolute quantities of the pheromone components D1 (46.65%) and D2 (41.40%) compared to the percentage of D1 in the pheromone blend (17.19%). This possibly suggests that *R. dominica* males feeding on the same host, vary in the production of quantity of the

pheromone but the ratio of the two pheromone components does not change much. Birgersson (1989) reported that the ratio of the pheromone components (pinene alcohols) was almost constant in males of the bark beetle, *Ips typographus* boring in the same tree but varied between males from different trees.

The considerable individual variation among beetles of the same age, feeding on the same host-grain under similar conditions indicates that the variation in pheromone production among individuals of *R. dominica* may be at least partially genetic. The genetic component of variation in pheromone production has also been reported for some other insects such as European corn borer (Klun and Maini, 1979) and bark beetle, *Ips typographus* (Birgersson *et al.*, 1988). However, external factors can also cause a variation in the pheromone production among individuals (see Chapter 6 & 7 for details). In the present studies, holes were drilled into the grains (to put beetles in) from the same side of the grains and the length of the holes was also approximately the same. However, there is a possibility that individual beetles may have bored into different parts, and during the entrainment period may be feeding on different parts of the grain. The nutritional value of various grain components can be different (Landry and Moureaux, 1980), therefore, there is a possibility that different beetles may be feeding in zones of different nutritional value. In connection with this it is worth noting that Mayhew and Phillips (1994) speculated that the quantity of pheromone produced by *R. dominica* males is associated with the nutritional value of the food.

5.4.2. Association between the pheromone signalling of *R. dominica* and its feeding/boring activity

The results of the present studies have shown that the quantity of dust produced by the beetles during feeding/boring in the grains was positively correlated with the total quantity of the pheromone and both of its components D1 and D2. However, there was no correlation apparent between ratio of the pheromone components in the blend and dust produced. Elkinton *et al.* (1980) reported that the amount of feeding and boring activity by the bark beetle, *Ips paraconfusus* in non-host plants was less than in host plants and also a lower quantity of pheromone was produced. The positive correlation between the quantity of pheromone produced by beetles and quantity of dust produced during feeding/boring suggests that feeding does not simply trigger pheromone production in *R. dominica*, but rather these may be a more complex relationship. The beetles may use some chemical(s) from hosts as a precursor(s) of pheromone biosynthesis. Bark beetles of *Ips* spp. produce their aggregation pheromone when they were exposed to vapours of pheromone precursor myrcene, a monoterpene present in their host trees (Hughes, 1974). Exposure to increased concentrations of vapours of precursor, increased the production of pheromone in bark beetles, *Ips paraconfusus.* In natural conditions this precursor would enter a beetle via its digestive tract (Byers, 1981). Thus the quantity of pheromone produced would depend on the concentration of precursor in the host (Birgersson, 1989) or rate of feeding.

5.4.3. Association between the body weight of *R. dominica* and its pheromone output or rate of feeding/boring

The quantity of dust produced was positively correlated with weight of the beetles recorded before the pheromone entrainments but not with the weight recorded after the entrainments. The weights of the beetles recorded before and after the pheromone entrainments were positively correlated with the quantity of pheromone component D1 and total quantity (D1+D2) but not with quantity of D2 or percentage of D1 in the blend. Similarly Miller and Roelofs (1980) did not find any significant relationship between body weight and quantity or component ratio of pheromone produced by red-banded leafroller moth, *Argyrotaenia velutinana* (Walker).

The quantity of the dust produced has proved to be more reliable and simple parameter to measure feeding/boring activity than the grain weight loss, as it can be measured more accurately compared to a very small change in a much larger weight of grain. The attempts to measure accurately the changes that occurred in the weight of grains due to feeding of beetles were hampered by the complex nature of the experiment. The reduction in the weight of grains can be caused by factors other than feeding/boring e.g. loss or gain of moisture content of grain etc. Although the grains were selected carefully so that grains of similar size and shape are used in the experiment, there may be differences between individual grains in their tendencies to gain or lose moisture. Therefore a slight difference in the rate at which moisture was lost from the grains during entrainment period could have affected the actual weight lost due to feeding of beetles. Future studies attempting

to measure changes in the weight of grains due to the feeding/boring of a single beetle should take into account all the factors that can affect the weight of grain, such as change in moisture content due to high or low humidity during the experimental period.

In conclusion, these studies have demonstrated that *R. dominica* males show considerable individual variation in the quantities of pheromone produced, and these quantities are positively correlated with their feeding/boring rate. The ratio of the pheromone components is not as variable as the absolute quantities of pheromone released under similar situations and is also not correlated with the feeding/boring rate or beetle body weight.

Chapter:6

Does host-type affect the nature of pheromone signalling by *R. dominica* males?

6.1. INTRODUCTION

The adults of *R. dominica* showed less "attraction" to volatiles from hosts wheat or maize than those from non-host groundnut (see Chapter 3). However, attraction to wheat or maize volatiles compared to groundnut volatiles was increased when the male beetles were also present on the host-grain (see Chapter 4). This demonstrated that the presence of male beetles on host-grain affects the comparative response of conspecific individuals towards those host-grain, and hence affects the process of host selection. It also indicates that, either *R. dominica* males do not produce their aggregation pheromone when present on a non-host or unsuitable host, or the pheromone signals they produce on host-grains of different types or suitability are different. It has been suggested that host plants may influence pheromone signalling by insects (Landolt and Phillips, 1997). There appear to be no previous studies on pheromone production by *R. dominica* or other stored insect pests feeding on unsuitable hosts or non-hosts. However, a few studies have been made of other insects. Elkinton *et al.* (1980) reported that the pheromone production and the amount of boring by the bark beetle, *Ips paraconfusus* L. was much less in white fir (non-host) than in ponderosa pine (host). Chang *et al.* (1988) reported that significantly greater quantity of pheromone was produced by boll weevil, *Anthonomus grandis* Boheman, feeding

on flower buds from glanded genotypes of cotton (i.e. genotypes with epidermal glands containing terpenoid aldehydes) than on glandless genotypes. The ratio of the pheromone components produced on both the genotypes was also different.

In this chapter, the effect of host-type on production of pheromone signals by *R. dominica* males and responses of males and females to those pheromone signals were studied. The pheromone production from single *R. dominica* males present in wheat, maize, groundnut or de-oiled groundnut kernels was quantified. This made it possible to determine whether any change(s) occurs in pheromone signals due to beetles being present in different types of host-grains, and whether pheromone signals are honest indicators of host quality/suitability. At the same time olfactometer tests were undertaken to observe the behavioural responses of the beetles towards these pheromone signals to investigate the effect of these changes on the responding beetles. In this way it was possible not only to demonstrate which treatment is more attractive but to explore which factors of a pheromone signal are responsible for the observed differences in response. The experimental procedure adopted also allowed an assessment of whether the differences in pheromone quantity released by different male signallers, as detected by gas-liquid chromatography analysis, were sufficient to affect the behavioural response observed in the olfactometer.

The studies were set out to address the following main questions:

- Do *R. dominica* males produce their pheromone signals at all when present in an unsuitable or non-host?

- If the pheromone is produced when present in an unsuitable or non-host, does it differ in quantity or quality from that produced in a suitable host?
- If the pheromone signals are modified when the beetle is in an unsuitable host, is this change reversible (i.e. does the signal revert to normal when the male is returned to a suitable host)?
- Is there any difference in the responses of the beetles towards volatiles from males present in different host-types?
- If there is a difference in the behaviour of beetles towards male signallers present in different host-types, which characteristics of the pheromone signal are most correlated to response, absolute quantities of pheromone components D1, D2 or ratio of D1 to D2?
- Is there any difference between the responses of males and females to these signals?

6.2. MATERIALS AND METHODS

Insects

R. dominica adults were removed from culture within twenty hours after emergence, sexed (see section 2.3) and kept singly on kibbled wheat grains for three days before transferring them into different host grains.

Beetles used to observe responses in olfactometer tests were sexed and kept singly on kibbled wheat grains for seven days before they were tested.

Pheromone entrainment

Details of the equipment used in pheromone entrainments and their arrangement is presented in section 2.5.

Olfactometer test

Details of olfactometer apparatus and procedures for recording observations of behavioural responses of beetles are given in section 2.4.

Statistical analysis

See section 2.6 for statistical methods used for analysis of data.

6.2.1. Pheromone production by *R. dominica* males present in wheat, maize, groundnut or de-oiled groundnut

Experimental Procedure

Single males were transferred into the previously prepared grains of wheat, maize, groundnut or de-oiled groundnut with twenty replications of each treatment. The grains were prepared by drilling approximately 4mm deep holes in them with a 1.5mm diameter drill. The beetles were moved into the holes drilled in the grains to make sure that all the beetles started to feed (bore) at the same time.

The males were allowed to feed in the grains for four days. On the fifth day the males in all the treatments were transferred to the pheromone entrainment chambers with airflow on but collection of pheromone was not started until twenty-four hours later and then continued for a further twenty four hours. Nine

entrainments were made every day, which included two repeats of each treatment and one control (without any insect or grain). The experiment lasted ten days, giving a total of twenty replications of each treatment. The experiment was set up in a way that all the beetles were of the same age at the time of pheromone entrainments. One replication each for groundnut and de-oiled groundnut was not included in the statistical analysis as no pheromone was detected.

6.2.2. Production of pheromone signals by single *R. dominica* males present in maize or groundnut and the response of conspecific males and females to those signals

Experimental procedure

The procedure adopted for this experiment is presented in (Figure 6.1). The single males were transferred into the previously prepared maize grains or groundnut kernels with ten replications of each treatment. The grains/kernels were prepared by drilling approximately 4 mm deep holes in them with a 1.5 mm diameter drill. The beetles were moved into the holes drilled in the grains to make sure that all the beetles started to feed (bore) at the same time.

The beetles were allowed to feed in the grains four days before the first set of pheromone entrainments. One half (five) of the replications of each treatment was used to collect the pheromone and the other half was used as the source of pheromone signals in olfactometer to observe the response of the conspecific males and females towards these treatments. Next day, the first half of the replications (which was used for pheromone entrainment the previous day) was used as

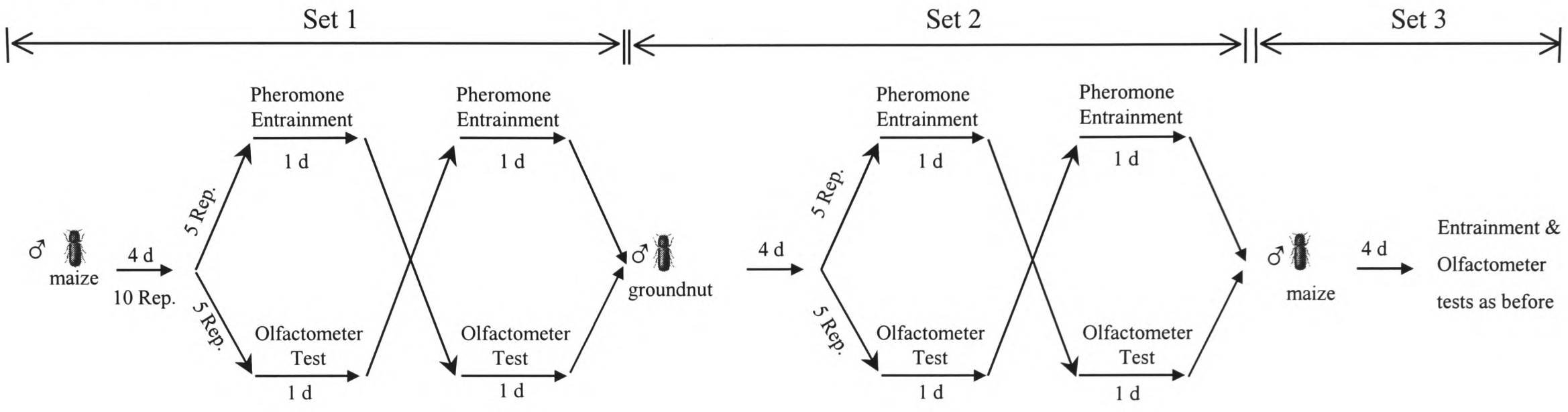


Figure 6.1: Protocol for pheromone entrainments and olfactometer tests showing both treatments i.e. males in maize grains and males in groundnut kernels

NB. d = day

pheromone source to test response of conspecifics in olfactometer and the second half (which was used for response experiment the previous day) was used for pheromone entrainment. Then the males in both the treatments were interchanged (the males which were present in maize grains were removed from the maize grains and were transferred into the groundnut kernels, and the males which were present in the groundnut kernels were transferred to the maize grains) and kept for four more days before performing the pheromone entrainment and olfactometer tests again. After completing the second set of pheromone entrainment and olfactometer experiments males were once again interchanged between the treatments before the third and final set of tests.

In each set of olfactometer tests, the responses of forty male and forty female beetles (four males and four females for each replication of treatments) were observed. The order in which the airflows were connected to the olfactometer exposure chamber changed after testing every ten males and ten females (i.e. after every $^1/_4$ of the olfactometer tests).

Statistical analysis

Spearman's rank test was used, to test for a correlation between differences in the quantities of the D1 and D2 between 1st & 2nd and 2nd & 3rd set of entrainments to investigate whether the magnitude of change in the quantities of pheromone components was consistent. The same test was used to correlate different characteristics of the pheromone signal with the response of the beetles in the olfactometer.

6.3. RESULTS

6.3.1. Pheromone production by *R. dominica* males present in wheat, maize, groundnut or de-oiled groundnut

The mean quantity of the pheromone component Dominicalure-2 (D2) produced during 24-hour period by males present in wheat or maize grains was slightly less than those present in groundnut or de-oiled groundnut kernels (Figure 6.2). The mean quantity of the pheromone component D1 produced by males present in wheat or maize grains was, however, greater than that produced by males present in groundnut or de-oiled groundnut kernels. The proportion of D1 in the blend was, therefore, greater for males present in wheat or maize grains than those present in groundnut or de-oiled groundnut kernels (Table-6.1).

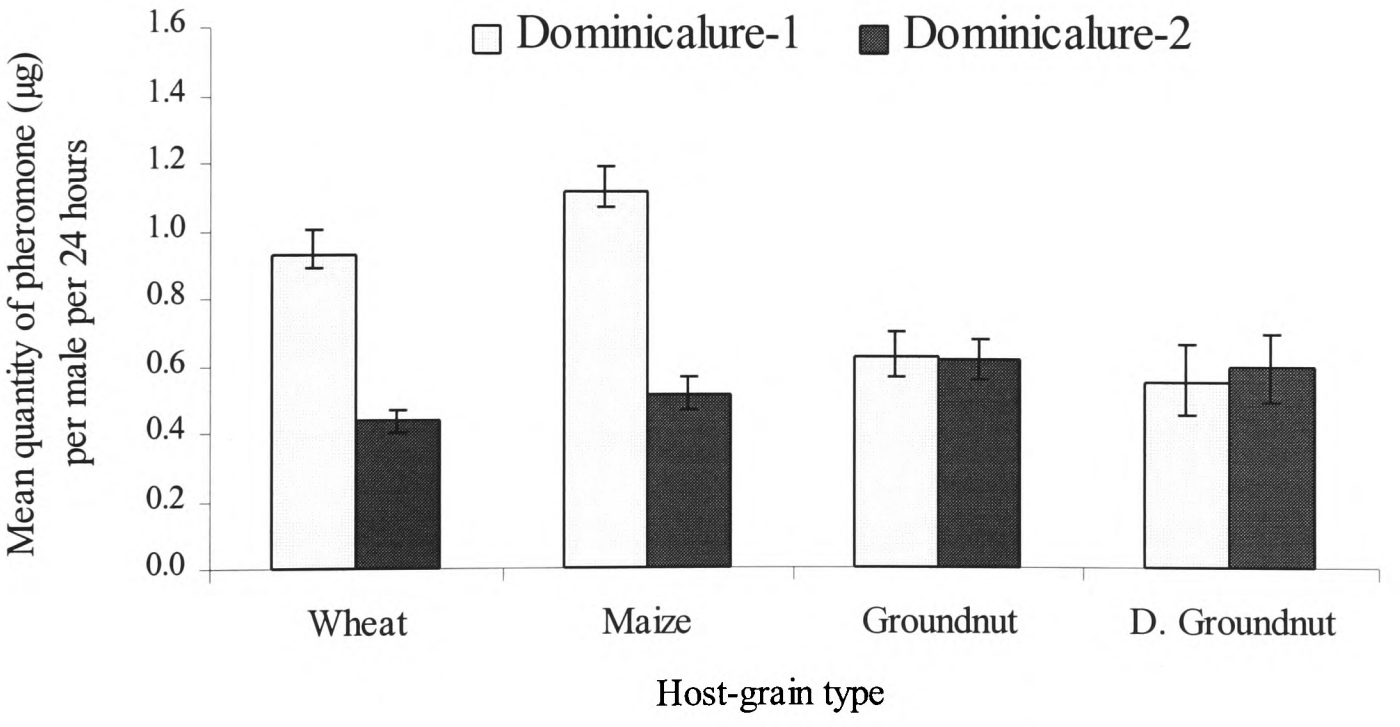


Figure 6.2: Mean quantities of the pheromone components Dominicalure-1 and Dominicalure-2 produced by the single *R. dominica* males present in different host grains; error bars represent standard error (n=20)
NB. De-oiled G'nut = De-oiled Groundnut

Table-6.1: Mean total quantity of Dominicalure-1 and Dominicalure-2 (µg) and percentage of the pheromone component Dominicalure-1 (D1) in the blend produced by single *R. dominica* males present in different host grains during a 24-hour entrainment (n=20)

Parameter	Wheat	Maize	Groundnut	De-oiled Groundnut
D1+D2 (µg)	1.36	1.63	1.25	1.15
	(0.46)	(0.44)	(0.558)	(0.92)
%D1	66.75	68.29	48.99	48.83
	(7.24)	(10.85)	(9.51)	(13.51)

NB. Standard deviation in parenthesis

Statistical analysis of the data showed significant difference between the quantities of D1 released by males present in different host grains (Table-6.2) while the total quantities of the pheromone (D1+D2) and component D2 were not significantly different between the treatments. Subsequent pairwise comparison between different treatments showed that quantity of the D1 produced by males present in wheat or maize was significantly greater than by males present in groundnut or de-oiled groundnut (Table-6.3). The percentage of the D1 in the pheromone blend was significantly different among different treatments (Table-6.2). Subsequent pairwise comparison between different treatments showed that the percentage of D1 in the pheromone blend produced by males in wheat or maize was significantly greater than by males present in groundnut or de-oiled groundnut (Table-6.3).

Table-6.2: One-way analysis of variance for quantities of pheromone components, Dominicalure-1 (D1), Dominicalure-2 (D2), total quantity (D1+D2), and percentage of D1 in the blend produced by single *R. dominica* males present in different host grains, wheat, maize, groundnut or de-oiled groundnut

Variable	D.F.	Sum of Sq.	Mean Sq.	F-Ratio	F-Prob.
D1	3	4.06	1.35	9.97	<0.001
D2	3	0.40	0.13	1.58	0.20
D1+D2	3	2.61	0.87	2.27	0.09
%D1	3	7789.27	2596.42	35.24	<0.001

Table-6.3: Pairwise comparison between different treatments for (A) the quantity and (B) the percentage of Dominicalure-1 in the pheromone blend, using Least Significant Difference test (LSD) ('*' indicates significant difference and '+' indicates non-significant difference)

Treatments	Wheat		Maize		Groundnut	
	(A)	(B)	(A)	(B)	(A)	(B)
Maize	+	+				
Groundnut	*	*	*	*		
D.G'nut*	*	*	*	*	+	+

NB. D.G'nut* = De-oiled Groundnut

6.3.2. Production of pheromone signals by single *R. dominica* males present in maize or groundnut and the response of conspecific males and females to those signals

Pheromone entrainment

- *Does host-type affect the nature of pheromone signals produced by males?*

In all three sets, the mean quantities of the pheromone components D1 and D2

produced in 24 hours by males present in maize grains were greater than those produced by males present in groundnut kernels (Figure 6.3). Likewise, the mean total quantities and the proportion of D1 released were greater for males present in maize grains (Table-6.4).

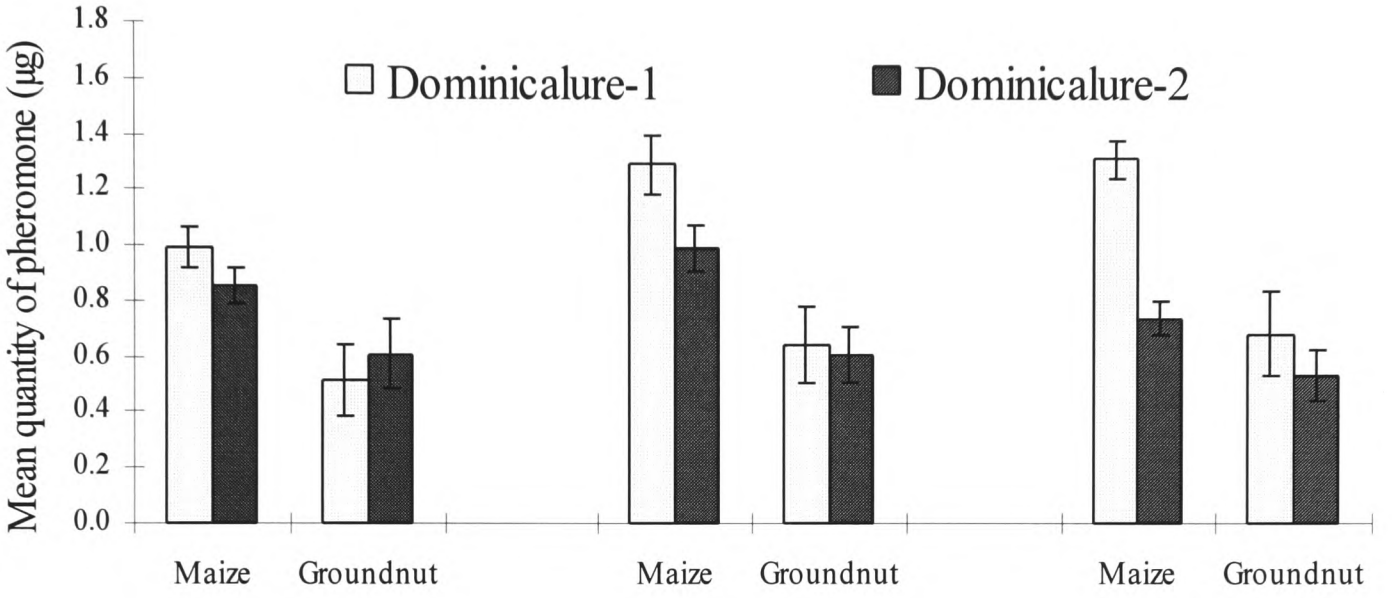


Figure 6.3: Mean quantities of pheromone components Dominicalure-1 and Dominicalure-2 produced by *R. dominica* males present in maize or groundnut in a 24-hour entrainment in the first, second and third set of tests; error bars represent standard error (n=10)

Table-6.4: Mean total quantities (µg) and percentage of pheromone component Dominicalure-1 (D1) in the blend produced by individual *R. dominica* males present in maize grains or groundnut kernels (n=10)

	First set		Second set		Third set	
Parameter	Maize	Groundnut	Maize	Groundnut	Maize	Groundnut
D1+D2 (µg)	1.84	1.12	2.28	1.24	2.05	1.22
	(0.36)	(0.78)	(0.55)	(0.74)	(0.27)	(0.75)
D1 (%)	53.71	44.79	56.57	50.55	64.13	54.54
	(7.24)	(4.79)	(4.11)	(5.80)	(7.52)	(7.32)

NB. Standard deviation in parenthesis

In the first and third set of entrainments there was a significant difference between treatments for the quantity of D1, total quantity of the pheromone and the ratio of the two components but the quantity of D2 was not significantly different (Table-6.5). In the second set of entrainments all the parameters, D1, D2, total quantity and ratio differed significantly between treatments (Table-6.5).

Table-6.5: Statistical comparison of the production of pheromone components Dominicalure-1 (D1) and Dominicalure-2 (D2), total quantity and percentage of Dominicalure-2 between males present in maize grains or groundnut kernels; data were analysed by independent samples t-test

	Variable	t-value	df	P-value (2-tailed)
1st set	D1	3.26	18	<0.01
	D2	1.75	18	0.10
	D1+D2	2.65	18	0.02
	%D1	3.25	18	<0.01
2nd set	D1	3.75	18	<0.001
	D2	2.96	18	<0.01
	D1+D2	3.55	18	<0.01
	%D1	2.68	18	0.02
3rd set	D1	3.80	18	<0.001
	D2	1.80	18	<0.09
	D1+D2	3.28	18	<0.01
	%D1	2.89	18	<0.01

- *Is the change in the pheromone signalling reversible?*

The pheromone outputs by individual males were studied to investigate whether a male beetle that showed a modification in pheromone signalling when transferred to another host, would restore 'normal' signalling when once again moved to the original host. The results demonstrated the reversible nature of the pheromone signalling when the test was initiated either with a male in a maize grain

(Figure 6.4a) or with a male in a groundnut kernel (Figure 6.4b). Change in the host effected the total quantity of the pheromone produced, but most of the observed effect came from change in the amount of pheromone component Dominicalure-1. In 65% and 85% of cases, respectively, greater quantities of pheromone components D1 and D2 were produced by the same males when they were present in maize grains than when they were present in groundnut kernels. It seems that the pheromone component Dominicalure-1 is more sensitive to the type of host than Dominicalure-2 (Figure 6.4a & b).

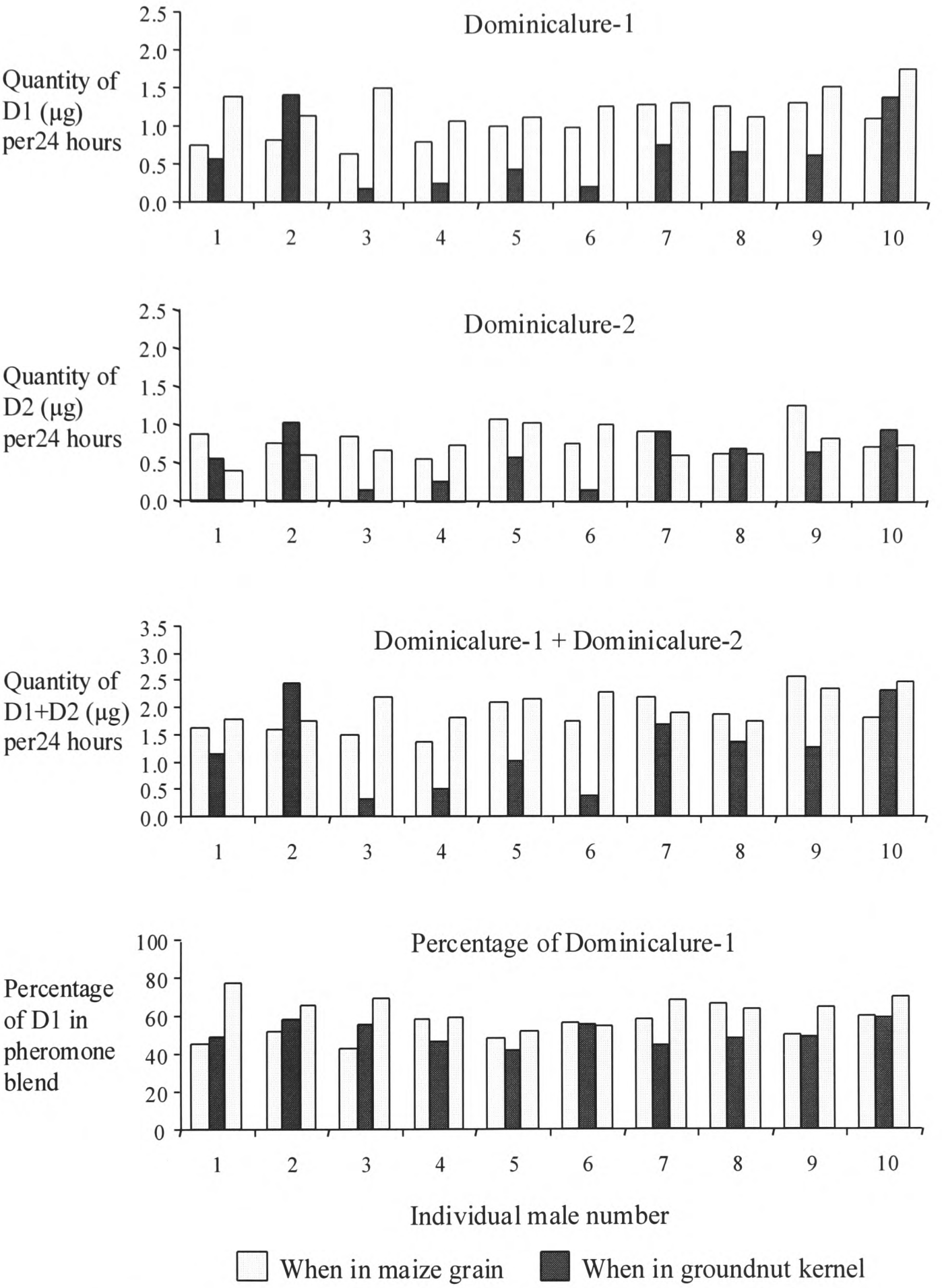


Figure 6.4a: Quantity of the pheromone components Dominicalure-1 (D1), Dominicalure-2 (D2), total quantity (D1+D2), and percentage of D1 in the pheromone blend produced by individual *R. dominica* males when present in maize grains or in groundnut kernels during twenty four-hour period; test was initiated with a male in a maize grain

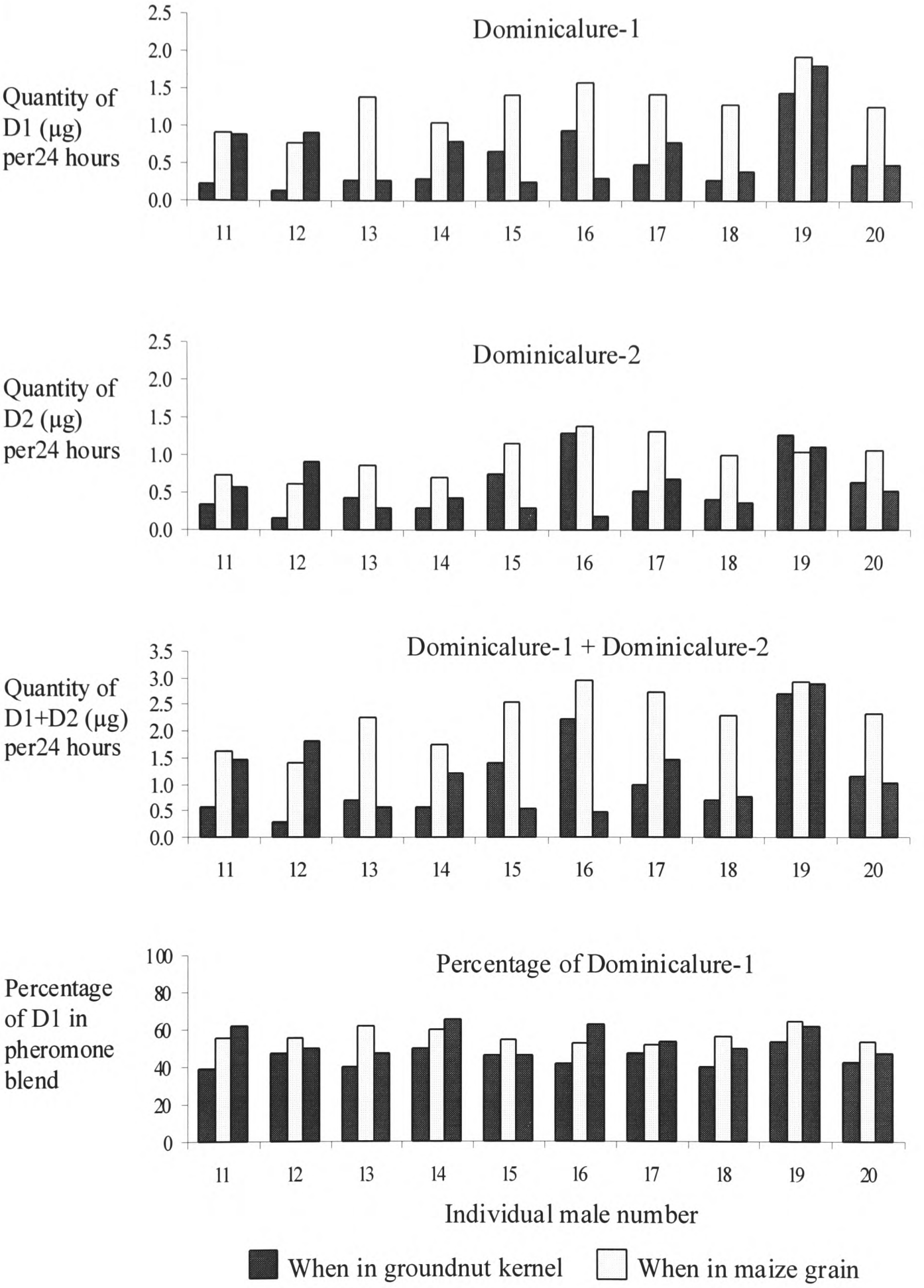


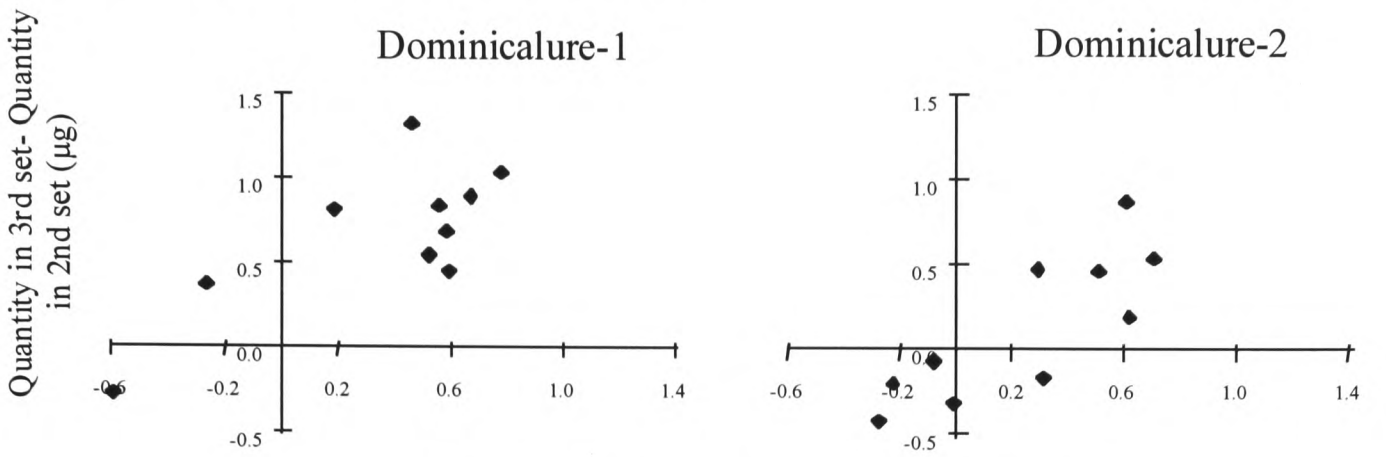
Figure 6.4b: Quantity of the pheromone components Dominicalure-1 (D1), Dominicalure-2 (D2), total quantity (D1+D2), and percentage of D1 in the pheromone blend produced by individual *R. dominica* males when present in maize grains or in groundnut kernels during twenty four-hour period; test was initiated with a male in a groundnut kernel

- *Is magnitude of change/modification in pheromone signalling by males due to the host-type consistent between hosts?*

To determine whether males are consistent in their sensitivity to change in host-type, the differences in the quantity of the pheromone components D1 and D2 (for the same beetle) between 1st & 2nd and 2nd & 3rd entrainments were plotted against each other. To calculate the difference between the sets, the quantity of pheromone produced in the case of males present in groundnut kernels was subtracted from that in case of males in maize grains. There was a significantly positive correlation between the sets for D1 ($r^2 = 0.81$, $p<0.01$, $n = 10$) and D2 ($r = 0.76$, $p=0.01$, $n = 10$) when the test was initiated with a male in a maize grain (Figure 6.5). No correlation in the changes between sets was apparent when test was initiated with a male in a groundnut kernel. However, as most values in the plots were positive it suggests that pheromone output in set 2 tended to be different from set 1 or set 3.

There was a significant positive correlation between pheromone components D1 and D2 when males were present on groundnut ($r^2 = 0.92$, $p<0.001$, $n = 30$) but there was apparently no correlation on maize ($r^2 = 0.29$, $p=1.15$, $n = 30$) (Figure 6.6).

a. Test initiated with a male in a maize grain:



b. Test initiated with a male in a groundnut kernel:

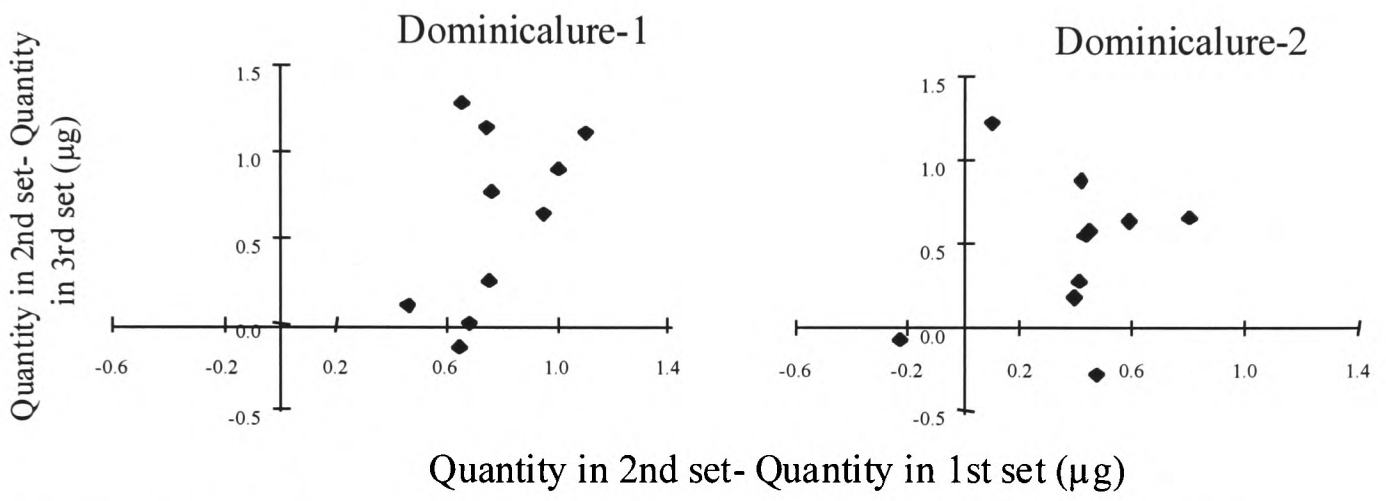


Figure 6.5: Correlation between the difference in the quantity of pheromone produced by the same beetle in first and second set of entrainments and the difference in quantity produced by the same beetle in the second and third set of entrainments

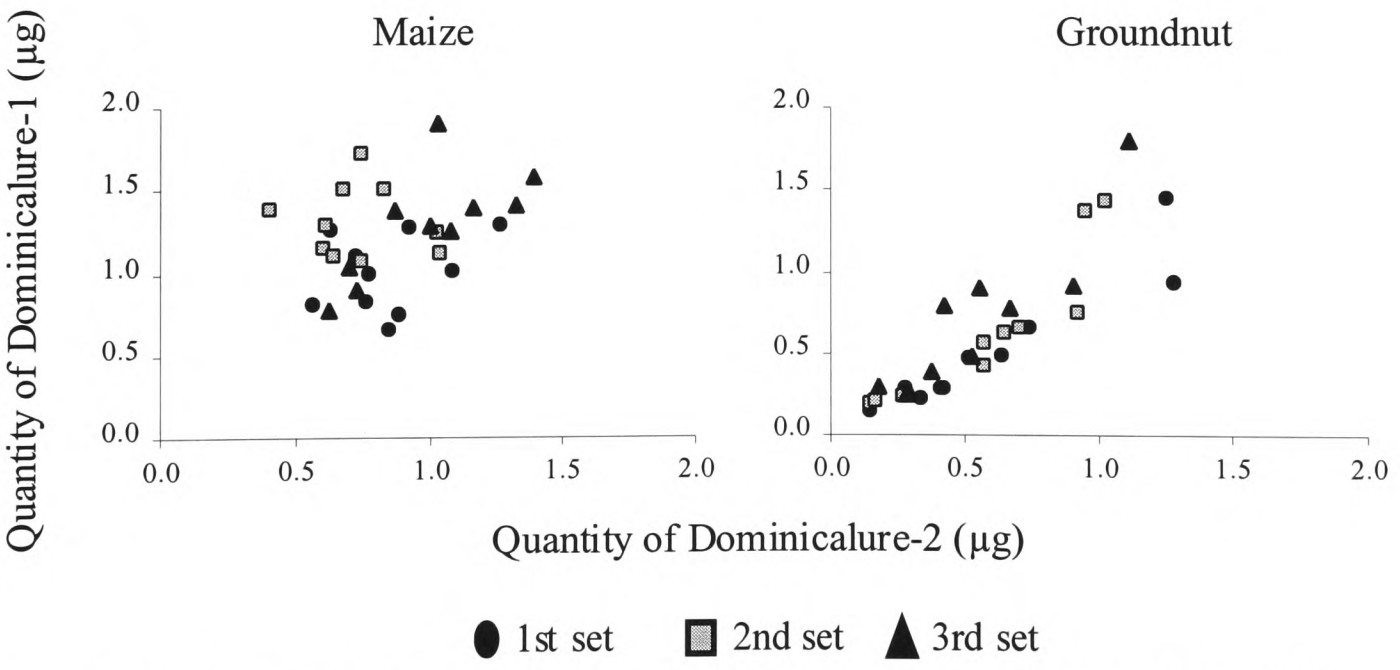


Figure 6.6: Correlation between quantities of pheromone components D1 and D2 produced in cases of males present in maize grains or in groundnut kernels

Olfactometer test

- *Is there any difference in the response of* R. dominica *to pheromone signals from males present in different host-types?*

In all three sets, adult *R. dominica* spent more time in the zone receiving volatiles from a male in a maize grain than the zone receiving volatiles form a male in a groundnut kernel or clean air (control zones) (Figure 6.7). There was significant heterogeneity among treatments for males [(Chi-Sq.= 10.44, df= 3, p=0.02), (Chi-Sq.= 9.44, df= 3, p=0.02) and (Chi-Sq.= 8.51, df= 3, p=0.04) for 1st, 2nd and 3rd test, respectively] and females [(Chi-Sq.= 29.74, df= 3, p<0.001, Chi-Sq.= 26.77, df= 3, p<0.001 and Chi-Sq.= 11.63, df= 3, p<0.01 for 1st, 2nd and 3rd test, respectively].

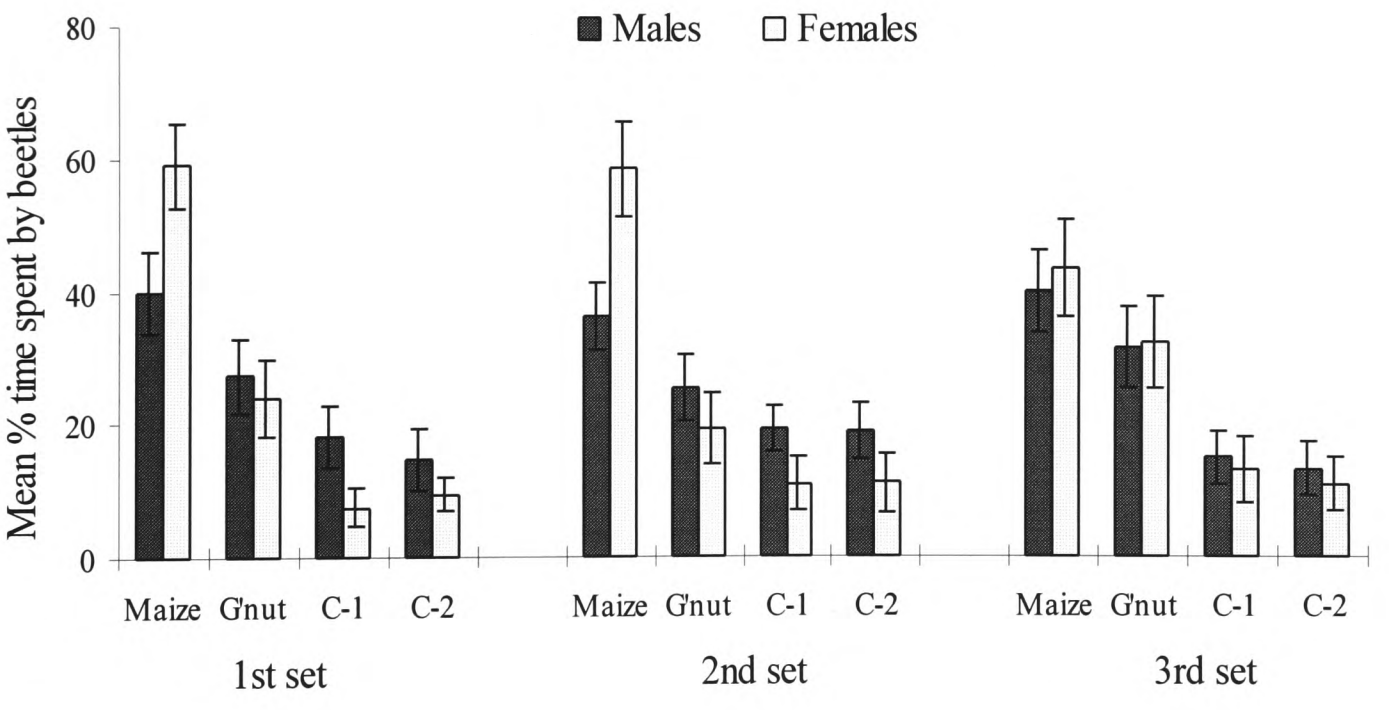


Figure 6.7: Mean percentage of time (5 minutes) spent by adult *R. dominica* in different treatment zones of a four-choice airflow olfactometer; the sources of odour tested were a male in maize grain and a male in a groundnut kernel; there were ten replications of each treatment (source of odour/volatiles) in each set and responses of four males and four females were observed for every one pair of treatments; error bars represent standard error
NB. G'nut = Groundnut; C = Control (clean air)

Subsequent pairwise analysis of the data between different treatments for each of the three sets showed that female responders were significantly more attracted to the male signallers present in maize grains than those present in groundnut kernels in first and second set (Table-6.6). However, female responders in the third set and male responders in all three sets showed no significant differences between their responses to male signallers present in maize grains or groundnut kernels (Table-6.6).

Table-6.6: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from *R. dominica* males present in maize or groundnut, or clean air (control); data were analysed using Wilcoxon test for two-related samples

	Treatment combination	Males	Females
1st set	Maize & Groundnut	0.25	<0.01
	Maize & Control-1	0.03	<0.001
	Maize & Control-2	<0.01	<0.001
	Groundnut & Control-1	0.18	0.03
	Groundnut & Control-2	0.08	0.046
	Control-1 & Control-2	0.67	0.48
2nd set	Maize & Groundnut	0.20	<0.01
	Maize & Control-1	<0.01	<0.001
	Maize & Control-2	0.03	<0.001
	Groundnut & Control-1	0.55	0.18
	Groundnut & Control-2	0.24	0.26
	Control-1 & Control-2	0.82	0.63
3rd set	Maize & Groundnut	0.46	0.50
	Maize & Control-1	<0.001	<0.01
	Maize & Control-2	<0.01	<0.001
	Groundnut & Control-1	0.03	0.03
	Groundnut & Control-2	0.06	0.03
	Control-1 & Control-2	0.91	0.78

The total number of males and females favouring different zones of test arena in all three sets combined, showed a clear stronger attraction for the volatiles released by males in maize grains than those in groundnut kernels. The percentage of time spent by each beetle in different zones also suggests the same (Table-6.7).

Table-6.7: In all three sets combined, total number of males and females favouring different zones of test arena in the four choice airflow olfactometer and (in parenthesis) mean percentage of time spent by beetles in those zones

	Maize	Groundnut	Control-1	Control-2
Males	51 (38.68%)	34 (28.18%)	16 (17.49%)	19 (15.65%)
Females	72 (53.77%)	29 (25.24%)	10 (10.52%)	9 (10.47%)

- *Is there any difference between the responses of males and females?*

a. Do male and female responders show the same level of response to odour sources?

The response of males or females to both sources of volatiles (i.e. male signallers present in maize grains and male signallers present in groundnut kernels) combined, showed that the females responded more strongly than males to the odour sources. The female response was significantly greater than male response for the second set ($z = -3.26$, $p<0.001$, $n = 40$) and the third set ($z = -1.96$, $p = 0.05$, $n = 40$). The female response (83%) was greater than males (67%) in the first set also but the difference was not significant.

b. Do male and female responders differ in their response to the same odour source?

The females showed significantly greater response than males to volatiles from males present in maize grains in the first ($z = -2.05$, $p = 0.04$, $n = 40$) and second ($z = -2.03$, $p = 0.04$, $n = 40$) set. However, there was no significant difference in response between males and females in the third set. Females response to males in groundnut kernels was significantly less than males ($z = -2.11$, $p = 0.04$, $n = 40$). However, there was not significant difference for first or third set.

c. Do male and female responders show same level of discrimination between odour sources?

The females showed higher level of discrimination between the male signallers present in two types of host-grains (86%, 83% & 83%, respectively) than males (70%, 68% & 80%, respectively) in first, second and third set, respectively. The differences were significant for first ($z = -2.75$, $p < 0.01$, $n = 40$) and second set ($z = -2.58$, $p = 0.01$, $n = 40$) but there was no significant difference for third set.

- *Which characteristic(s) of the pheromone signal are most correlated to response?*

There may be at least four important factors in the pheromone blend of *R. dominica* i.e. quantity of pheromone component D1, quantity of pheromone component D2, total quantity of both components and ratio of the components D1 and D2. In order to investigate which factor might be responsible for observed responses in bioassay, comparisons were made between the chemical data and the behavioural

data available for each pair of male signallers. The preference of the responding beetles given the choice of two signals was calculated using the formula below.

Response to male in maize grain (% time spent in the zone)	-	Response to male in groundnut kernel (% time spent in the zone)

The average of the percentage response of four beetles tested for the same male signaller was calculated (separately for males and females) and plotted against different possible factors affecting the response of the beetles towards a volatile source. These different factors were:

D1 released by male in maize grain	-	D1 released by male in groundnut kernel
D2 released by male in maize grain	-	D2 produced by male in groundnut kernel
D1-D2 produced by male in maize grain	-	D1-D2 released by male in groundnut kernel
% D1 in the blend released by males in maize grains	-	% D1 in the blend released by males in groundnut kernels

There was no strong correlation between any of these factors and response of the beetles. An example of the data is given in Figure 6.8.

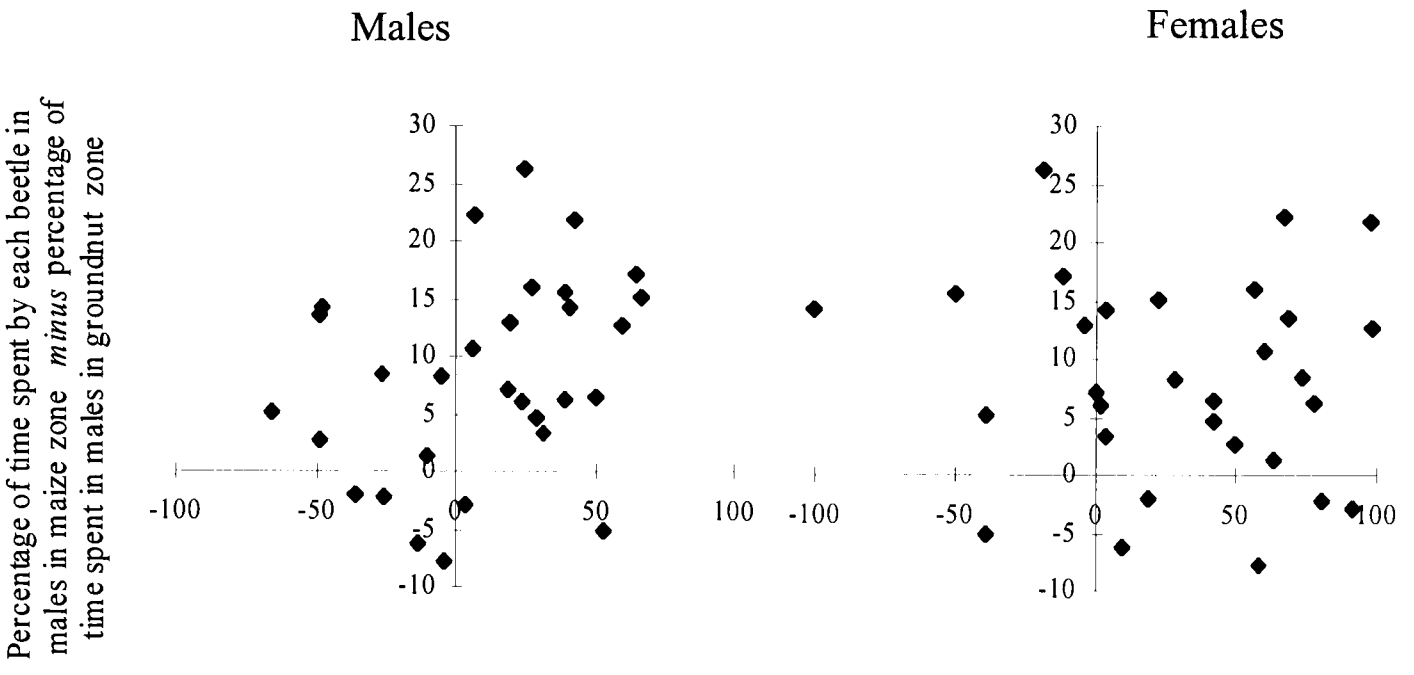


Figure 6.8: Correlation between the preference of male or female beetles for the zones of a test arena receiving volatiles from males in maize grain or in groundnut kernels and the difference in %D1 in the pheromone blends released by these beetles

6.4. DISCUSSION

The results demonstrate that *R. dominica* males would produce pheromone signals even when present in an unsuitable host (groundnut in this case). However, the pheromone signals produced by males on this unsuitable host differed from those produced on a suitable host (maize). The results of the first experiment (section 6.3.1) showed that the quantity of pheromone component D2 was not affected by the host-grain type, but the quantity of D1 produced by males present in groundnut or de-oiled groundnut kernels was significantly smaller than that produced by males present in wheat or maize. This affected the ratio of the two components in the pheromone blend, and resulted in significantly smaller proportion of D1 in the blend released by males present in groundnut or de-oiled groundnut kernels than

that released by males in wheat or maize. The results of the second experiment (section 6.3.2.) confirm that pheromone component D1 is more sensitive to the type of host-grain than D2. In the second experiment, in all three sets of pheromone entrainments, the major effect was on the quantity of D1 although the quantity of D2 was also affected by the host-grain type. The mean quantities of D1 produced by males present in groundnut kernels were 48, 50 and 47 percent less than those they produced in maize grains in first, second and third set of entrainments, respectively, compared to 29, 39 and 27 percent of D2.

Study of the pheromone output by individual males showed that generally the change in the pheromone signals due to the type of host-grain is reversible (Figure 6.4a & 6.4b). However, no consistency in the magnitude of the change in pheromone signals between host-types was observed.

The olfactometer test was designed to determine the preference of the beetles between the volatiles when males are on an unsuitable host (groundnut) and to those released when males are on a suitable host (maize). The results showed that in all three sets of olfactometer tests, beetles preferred volatiles when males were present in maize grains to those when males were in groundnut kernels. However, male responders did not differ significantly in their response to signallers present in maize grain or groundnut kernels. In contrast, female responders showed significant greater response to signallers present in maize grains than to those present in groundnut kernels, in the first two sets but not in the third set. It should be noted that the males used as a source of pheromone signals in the olfactometer bioassay tests were present in maize grains or groundnut kernels, and groundnut on

its own is more attractive than maize (see Chapter 3).

Response of the beetles in the olfactometer was not statistically significantly correlated to the different characteristics of the pheromone blend. Within the confines of the olfactometer test it is very difficult to determine whether the ratio or the actual quantity of the components (especially D1) is more important in determining beetle's response, and to correlate those responses to the characteristics of a pheromone signal. But the results have shown males in groundnut are less attractive to conspecific individuals.

The stronger positive response of females to male signallers present in maize grains perhaps indicates that the original biological role of aggregation pheromone of *R. dominica* is sexual and females choose their potential mates on the basis of their ability to find a better food source. Females of related species *Prostephanus truncatus* also showed stronger attraction than males to male-produced aggregation pheromone in field experiments (Scholz *et al.*, 1997a, Hodges *et al.*, 1998). If the pheromone signal is the basis of selection of a potential mate by the females then it is difficult to understand why a male would make itself less attractive by releasing an inferior pheromone signal while present in an unsuitable host? Possibly beetles are restricted to give 'honest' signals, but variation in pheromone signals produced by individual males present in the same host (see Chapter 5 for details) suggests that some males are able to be more 'dishonest' than others.

The lower level of response from responding conspecific individuals to male signallers present in groundnut kernels than to those present in maize grain suggests that the pheromone signals possibly contain information about the

suitability of a food source. However, more hosts need to be tested to confirm this suggestion.

Generally, differences in the pheromone released by males in maize grains and males in groundnut kernels detected by gas-liquid chromatography analysis were successfully demonstrated by the olfactometer test. Capillary gas chromatography analysis showed greater quantities of pheromone, especially pheromone component D1, were produced by male signallers present in maize grains. In olfactometer tests, responding beetles showed greater response to the volatiles from males present in maize grain. However, when each pair of source and responding beetles were compared individually, the magnitude of the difference between the response of the beetles to male signallers present in maize grains or groundnut kernels did not correlate with the magnitude of the difference in the pheromone between two signallers. As it was not possible to collect the pheromone from the beetles and at the same time undertake a behavioural bioassay, these two activities were separated by one day. It was assumed that pheromone output would be similar on both these two days. The quantities of pheromone measured by gas-liquid chromatography analysis were collected over a twenty-four hour period at a rate of 1 litre air/minute while beetles in the olfactometer had five minutes to respond to the volatiles which they were receiving at a rate of 250cm^3 air per minute. This means, for a difference of 0.72μg (difference between the total quantities of pheromone released by male signallers present in maize grains and those present in groundnut kernels in first set) detected by gas-liquid chromatography analysis, beetles in the olfactometer discriminate an average difference in pheromone output of 0.0025μg. This very small difference may explain why response of beetles did not match exactly the

pheromone differences detected by chromatography analysis. However, the possibility that in some cases the beetles may be producing a different quantity or ratio of the pheromone in olfactometer test than they produced during pheromone entrainment although unlikely cannot be discounted.

In conclusion, males reduced the output of the pheromone blend when they were present in groundnut kernels and, the reduction was more noticeable for D1 than D2. Study of the pheromone output by individual beetles showed that in most of the cases higher quantities of pheromone were produced by the same beetles when they were present in maize grains and lower quantities were produced when they were present in groundnut kernels. The beetles preferred the volatiles coming from males in maize grains over those coming from males in groundnut kernels. Females were more strongly attracted than males to the volatiles from males present in maize grains. Similar differences between responses of males and females were reported in Chapter 4.

Chapter: 7

Does the presence of conspecifics affect the nature of pheromone signalling by *R. dominica* males?

7.1. INTRODUCTION

A general mode for aggregation pheromone function under natural conditions is that a male beetle would begin to release its pheromone after arriving at a suitable food source. Conspecific males and females would respond to these signals and aggregate at the food source. However, the arrival of new individuals should be inhibited at some stage to prevent over-crowding and over use of the food source. Some of the bark beetles have developed a unique system for this purpose, they release a repellent once mating pairs are established and an appropriate density is achieved (Borden, 1982). Other possible mechanisms to inhibit the arrival of new individuals can be to cease pheromone production or to produce a modified pheromone signal. Many insect species are known to cease pheromone production following mating particularly amongst the Lepidoptera (Raina and Menn, 1987). The rate of pheromone production by males of a species closely related to *R. dominica, Prostephanus truncatus* (Horn), is greatly reduced in the presence of live conspecific females or even on food previously infested with females (Smith *et al.*, 1996). Males of the bark beetle *Ips paraconfusus* Lanier reduce the rate of production of their aggregation pheromone as they are joined by females, and pheromone production ceases when the harem (3-5 females) is completed (Borden, 1967). The existence of any such system in *R. dominica* is not known. Mayhew

and Phillips (1994) reported that pheromone production per male in groups of 5 or 15 males was significantly lower than by single males. Increase in the quantity of the food did not increase the quantity of the pheromone in groups of males. The quantity of pheromone released by mated males (in the presence or absence of females) was not significantly different from unmated males of the same age. However, they suggested that, as the mating system of *R. dominica* is not fully understood it is not certain that the effect of mating on pheromone production was adequately tested by pairing one male with one female.

In the present studies, pheromone production by single *R. dominica* males in the presence of different numbers of females was quantified to investigate whether the presence of more than one female has any effect on pheromone signalling by males. Pheromone production by different sized groups of males was also quantified.

Investigation of the quantity and quality of pheromone production was coupled to the bioassay experiments, using a four-choice airflow olfactometer, to give a better understanding of the connection between the nature of the pheromone signal and the responses of beetles.

The basic questions asked in these studies were:

- Does the presence of conspecific females have any effect on the pheromone signals by males?
- If the pheromone signals are modified in the presence of females, is this change reversible?

- Is response of beetles to a modified pheromone signal different from the response to a normal pheromone signal?

- If there is a difference in the behavioural response of beetles towards two different male signallers, which characteristics of the pheromone signal are most correlated to response, absolute quantities of pheromone components D1, D2 or ratio of D1 to D2?

- Is there any difference in the response of males and females to pheromone signals?

- Does the pheromone production by males present in groups differ from that of single males?

7.2. MATERIALS AND METHODS

Insects

Adults were removed from the culture within twenty four hours of their emergence. After sexing (see section 2.3), beetles were kept singly for three days on kibbled wheat grain before being used in the experiments.

Beetles used to observe responses in olfactometer tests were sexed and kept singly on kibbled wheat grains for seven days before they were tested.

Pheromone entrainment

Details of the equipment used in pheromone entrainments and their arrangement is presented in section 2.5.

Olfactometer test

Details of olfactometer apparatus and procedures for recording observations of behavioural responses of beetles are given in section 2.4.

Statistical analysis

See section 2.6 for statistical methods used for analysis of data.

7.2.1. Effect of the presence of conspecific females on the nature of pheromone signalling by *R. dominica* males

Experimental procedure

The single males were confined with females in different treatments as follows:

- Lone male (Control)
- Male with one female
- Male with three females
- Male with five females
- Male with seven females

In each case, 1.5g of small pieces of maize of sizes between 1.7-3.4 mm were used as food. The quantity of food supplied was sufficient for the needs of the beetles as a large portion of food was still unconsumed even one week after the completion of the experiment. Males were kept for four days with the females, and on the fifth day the beetles in all the treatments were moved to the pheromone entrainment chambers (for detail and arrangement of pheromone equipment see section 2.5) with airflow on, but collection of pheromone was not started until twenty four

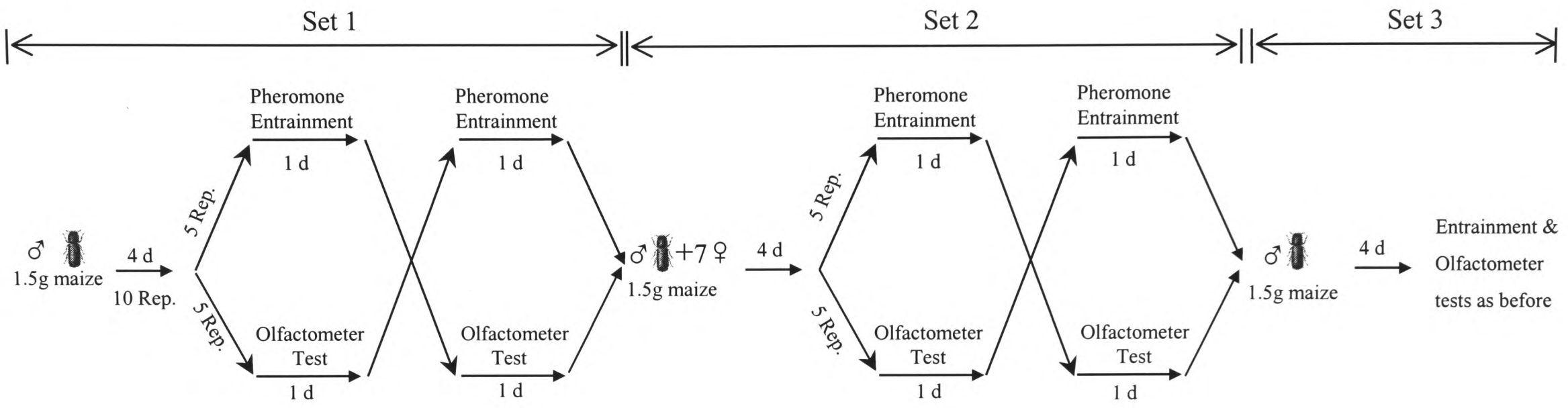
hours later and then continued for twenty four hours. Pheromone entrainments from two replicates of each treatment were made at a time and the experiment was continued for five days, making a total of ten replications of each treatment. The experiment was set up in a way that all the beetles were of the same age at the time of pheromone entrainments.

7.2.2. Production of pheromone signals by single *R. dominica* males in the presence of females and response of conspecific males and females to those signals

Experimental Procedure

The experiment included two treatments, in one treatment, all the males were kept alone while in the other, single males the were confined with seven females. In each case, 1.5g of small pieces of maize of sizes between 1.7-3.4 mm were used as food.

The pheromone was collected for a twenty four-hour period from lone males and males present with the females, with each of the treatments having ten replications (Figure 7.1). The beetles were put on food four days before the first set of pheromone entrainments. One half (five) of the replications of each treatment was used to collect the pheromone and the other half was used as the source of pheromone signals in the olfactometer to observe the response of the other beetles towards these treatments. Next day, the first half of the replications (which was used for pheromone entrainment the previous day) was used for olfactometer test and the second half (which was used in the olfactometer the previous day) was used



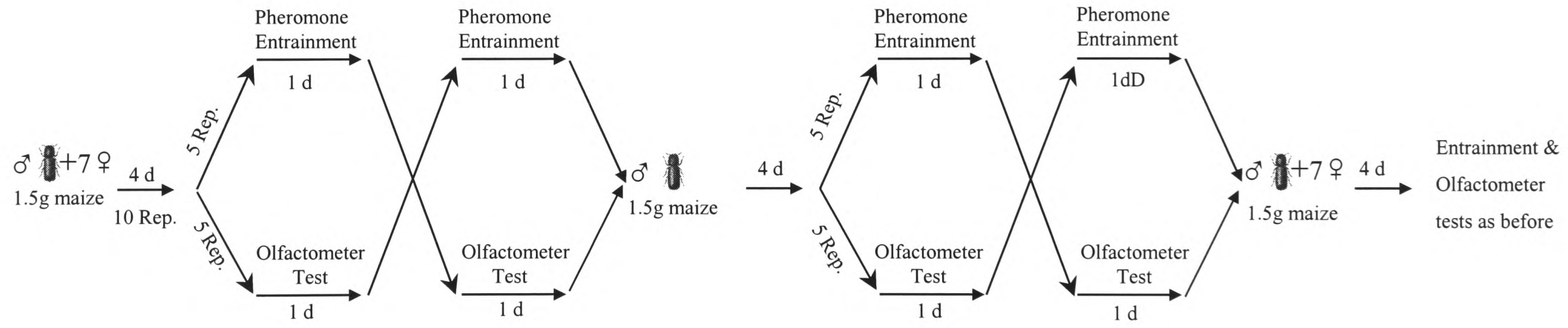


Figure 7.1: Protocol for pheromone entrainments and olfactometer tests showing both treatments i.e. alone male and a male present with seven females

NB. d = day

for pheromone entrainment. Then the males in both the treatments were interchanged (the single males which were present with the females were separated from the females and kept alone, and the males which were present alone were confined with the females) and kept for four more days before performing the pheromone entrainment and olfactometer tests again. After completing the second set of pheromone entrainment and olfactometer tests, male beetles were once again interchanged between the treatments before the third and final set of tests. After every set of tests when the males were interchanged between the treatments, beetles in both the treatments were put on fresh food (1.5 g of small pieces of maize).

In each set of olfactometer tests the responses of forty male and forty female beetles (four males & four females for each replication of treatments) were observed. The order in which the airflows were connected to the chamber was changed after testing every ten males and ten females (i.e. after every $^1/_4$ of the olfactometer tests).

Statistical analysis

Spearman's rank test was used to test for a correlation between differences in the quantities of the D1 and D2 between 1st & 2nd and 2nd & 3rd set of entrainments to investigate whether the magnitude of change in the quantities of pheromone components is consistent between individuals. The same test was used to correlate different characteristics of the pheromone signal with the response of the beetles in the olfactometer.

One replication of a male with seven females in the first set of pheromone

entrainments was not included in the statistical analysis as that male did not release any pheromone quantity detectable by chromatography analysis.

7.2.3. Effect of the presence of conspecific males on the nature of pheromone signalling by *R. dominica* males

Experimental procedure

The males were confined in different groups as follows:

- Single male (Control)
- Two males
- Four males
- Six males
- Eight males

In each case, 1.5g of small pieces of maize of sizes between 1.7-3.4 mm were used as food. Quantity of food supplied was sufficient for the beetles as a large portion of food was still unconsumed at the end of the experiment. Males were kept for four days in groups, on the fifth day they were moved to the pheromone entrainment chambers with airflow on but collection of pheromone was not started until twenty four hours later and then continued for a further twenty four hours. Pheromone entrainments from two replications of each treatment were made at a time and the experiment was continued for six days, making a total of twelve replications of each treatment.

7.3. RESULTS

7.3.1. Effect of the presence of conspecific females on the nature of pheromone signalling by *R. dominica* males

Similar mean quantities of the pheromone component Dominicalure-2 (D2) were produced during the 24 hour-period by males in all the treatments (Figure 7.2). However, the mean quantity of the pheromone component Dominicalure-1 (D1) produced by lone males was greater than that produced by males present with different numbers of females.

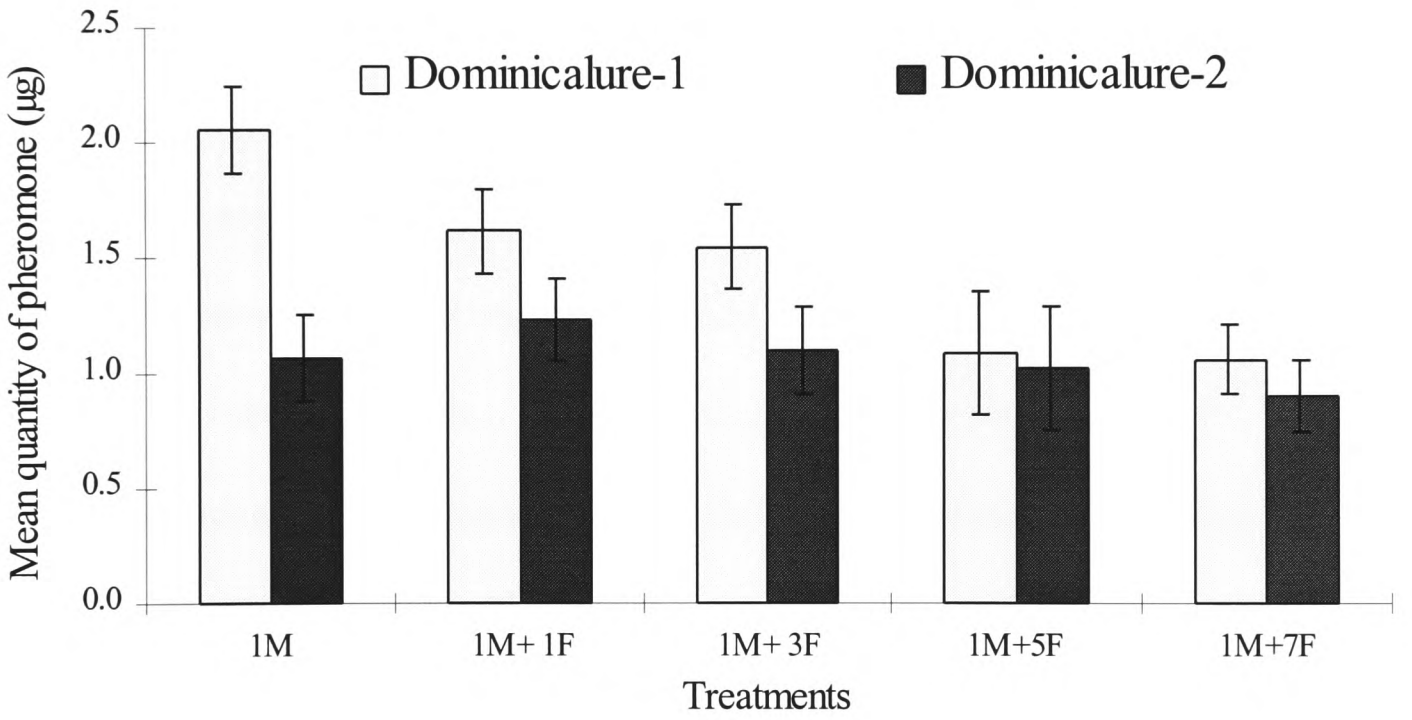


Figure 7.2: Mean quantities of the pheromone components Dominicalure-1 and Dominicalure-2 produced by the single *R. dominica* males present with different numbers of females; error bars represent standard error (n=10)
NB. M = Male; F = Female(s)

The mean total quantity of pheromone (D1+D2) decreased as the number of females confined with the single males increased (Table-7.1). The proportions of the two components in the total quantity was different in all the cases but the difference was less obvious in the presence of five or seven females (Figure 7.2).

Table-7.1: Mean total quantity of Dominicalure-1 and Dominicalure-2 (μg) and percentage of the pheromone component Dominicalure-1 (D1) in the blend produced by single *R. dominica* males present with different numbers of females (n=10)

Parameter	Lone male	Male with 1 female	Male with 3 females	Male with 5 females	Male with 7 females
D1+D2	3.12	2.85	2.65	2.11	1.96
	(1.62)	(1.17)	(1.64)	(1.72)	(1.19)
%D1	66.41	57.42	55.41	50.45	51.57
	(5.13)	(7.32)	(9.07)	(8.83)	(7.54)

NB. Standard deviation in parenthesis

Statistical analysis of the data showed no significant differences between the quantities of the pheromone components D1 or D2, and total quantities (D1+D2) released by males in different treatments. When percentage of the D1 in the pheromone blend was calculated and analysed, it showed statistically significant difference among different treatments (Table-7.2). Subsequent pairwise comparison between different treatments showed that the percentage of the D1 in the pheromone blend produced by lone males was significantly greater than by males present with females (Table-7.3).

Table-7.2: One-way analysis of variance for quantities of Dominicalure-1 (D1), Dominicalure-2 (D2), total quantity (D1+D2), and percentage of D1 in the blend produced by single *R. dominica* males present with different numbers of females

Variable	D.F.	Sum of Sq.	Mean Sq.	F-Ratio	F-Prob.
D1	4	6.88	1.72	2.07	0.10
D2	4	0.60	0.15	0.38	0.82
D1+D2	4	9.71	2.43	1.10	0.37
%D1	4	1609.36	402.40	6.78	<0.001

Table-7.3: Pairwise comparison between different treatments for the percentage of Dominicalure-1, using Duncan's multiple range test ('*' indicates significant difference and '+' indicates non-significant difference)

Treatments	1M	1M+1F	1M+3F	1M+5F
1M+1F	*			
1M+3F	*	+		
1M+5F	*	+	+	
1M+7F	*	+	+	+

NB. M = Male; F = Female(s)

7.3.2. Production of pheromone signals by single *R. dominica* males in the presence of females and response of conspecific males and females to those signals

Pheromone entrainment

- *Are pheromone signals modified in the presence of conspecific females?*

In all three sets, the mean quantities of D1 and D2 produced in twenty four hours by lone males were greater than those produced by males with females (Figure 7.3). Likewise, the mean total quantities and the proportion of D1 released were greater for lone males (Table-7.4). The ratio of the D1 to D2 varied between sets and between treatments. The proportion of D1 and overall amounts of pheromone in both the treatments tended to be lower in the first set than the other two.

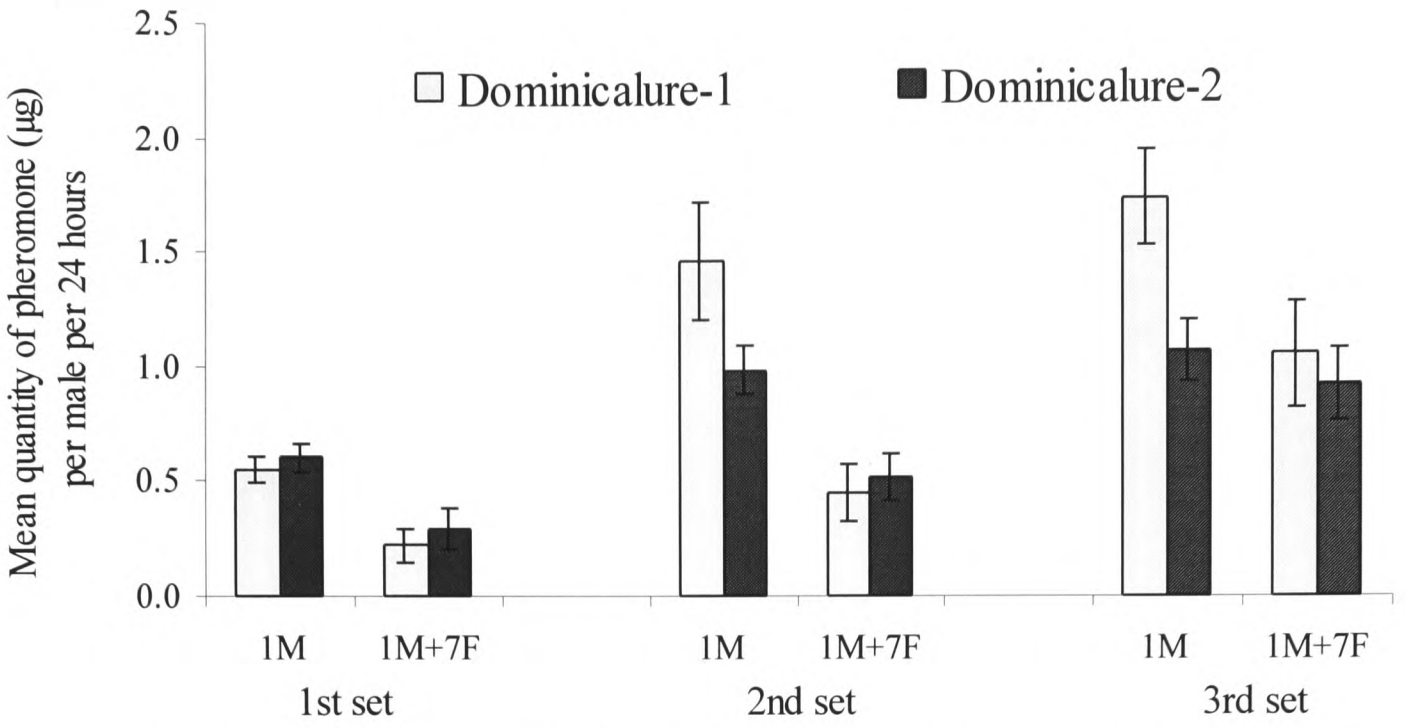


Figure 7.3: Mean quantities of the pheromone components Dominicalure-1 and Dominicalure-2 produced by *R. dominica* males present alone or with seven females during 24-hour period in first, second and third set of entrainments (n=10)
NB. M = Male; F = Females

Table-7.4: Mean total quantity of Dominicalure-1 and Dominicalure-2 (µg) and percentage of the Dominicalure-1 in the pheromone blend produced by individual *R. dominica* males present alone or with seven females (n=10)

	First set		Second set		Third set	
Parameter	Male alone	1Male+ 7Females	Male alone	1Male+ 7Females	Male alone	1Male+ 7Females
D1+D2 (µg)	1.15	0.51	2.44	0.97	2.80	1.97
	(0.35)	(0.51)	(1.16)	(0.69)	(1.19)	(1.05)
D1 (%)	48.02	43.25	59.87	46.20	61.82	53.25
	(6.60)	(6.93)	(7.85)	(7.78)	(4.67)	(8.18)

NB. Standard deviation in parenthesis

In the first set of pheromone entrainments there was a significant difference between treatments for the D1 and D2 releases but the ratio of the two components was not significantly different (Table-7.5). In the second set of entrainments both components and ratio differed significantly between treatments. In the third set,

output and ratio of the D1 differed significantly between the two treatments although D2 outputs were similar.

Table-7.5: Statistical comparison of the production of the pheromone components Dominicalure-1 and Dominicalure-2 and percentage of Dominicalure-1 between lone males and males with seven females; data were analysed by independent samples t-test

Entrainments	Variable	t-value	df	P-value (2-tailed)
1st set	D1	3.42	16	0.01
	D2	2.56	16	0.02
	%D1	0.95	16	0.35
2nd set	D1	3.51	18	<0.01
	D2	3.00	18	<0.01
	%D1	4.17	18	<0.001
3rd set	D1	2.23	18	0.04
	D2	0.73	18	0.48
	%D1	4.08	18	<0.001

- *Is the change in the pheromone signalling reversible?*

The pheromone outputs by individual males were studied to investigate whether a male beetle, that showed a modification in pheromone signalling when confined with females, will restore 'normal' signalling when separated from them and again kept alone or vice versa. The results demonstrated the reversible nature of the pheromone signalling in both the cases, when test was initiated with a male present alone (Figure 7.4a) or when test was initiated with a male present with females (Figure 7.4b). The presence of females affected the quantity of both the pheromone components, however, D1 was more affected than D2. In 80% of the cases, greater

total quantities of pheromone were produced by the same males when they were present alone and smaller quantities were produced when they were present with females. Likewise in 83 % of the cases, a greater percentage of the D1 in the blend was produced by the same males when they were present alone (Figure 7.4a & 7.4b).

In the previous Chapter (6) the characteristic of the pheromone signals of *R. dominica* males that was affected the most due the beetles present on an unsuitable host (groundnut) was quantity of pheromone component D1. However, in the present case the ratio of the two components (%D1 in the blend) seems the pheromone characteristic that has been affected the most due to the presence of females with the male signallers (Figure 7.4a & 7.4b).

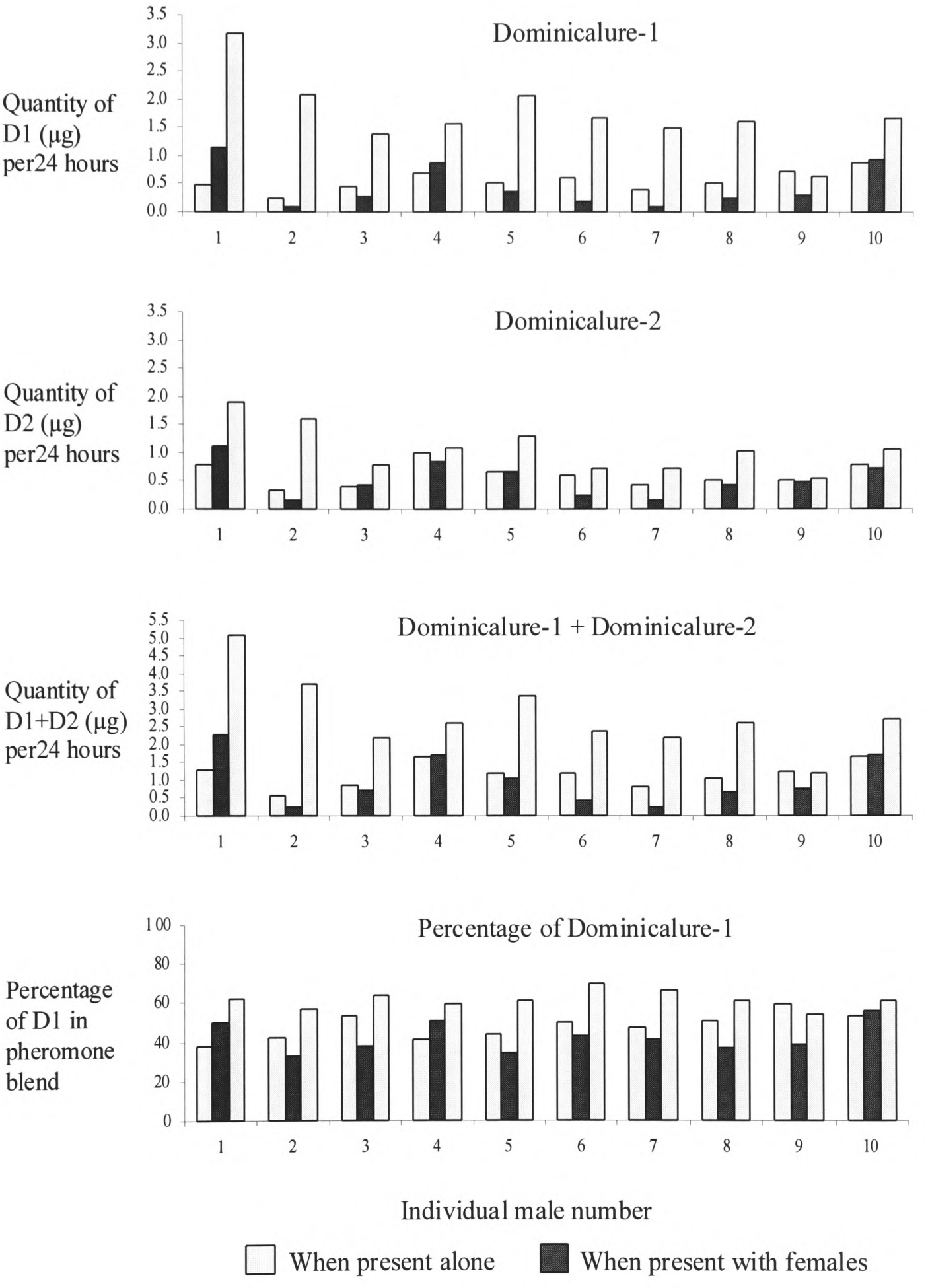


Figure 7.4a: Quantity (µg) of the pheromone components Dominicalure-1, Dominicalure-2, total quantity of both components, and percentage of Dominicalure-1 in the pheromone blend produced by individual *R. dominica* males when present alone or with seven females in twenty four hours; test was initiated with a male present alone

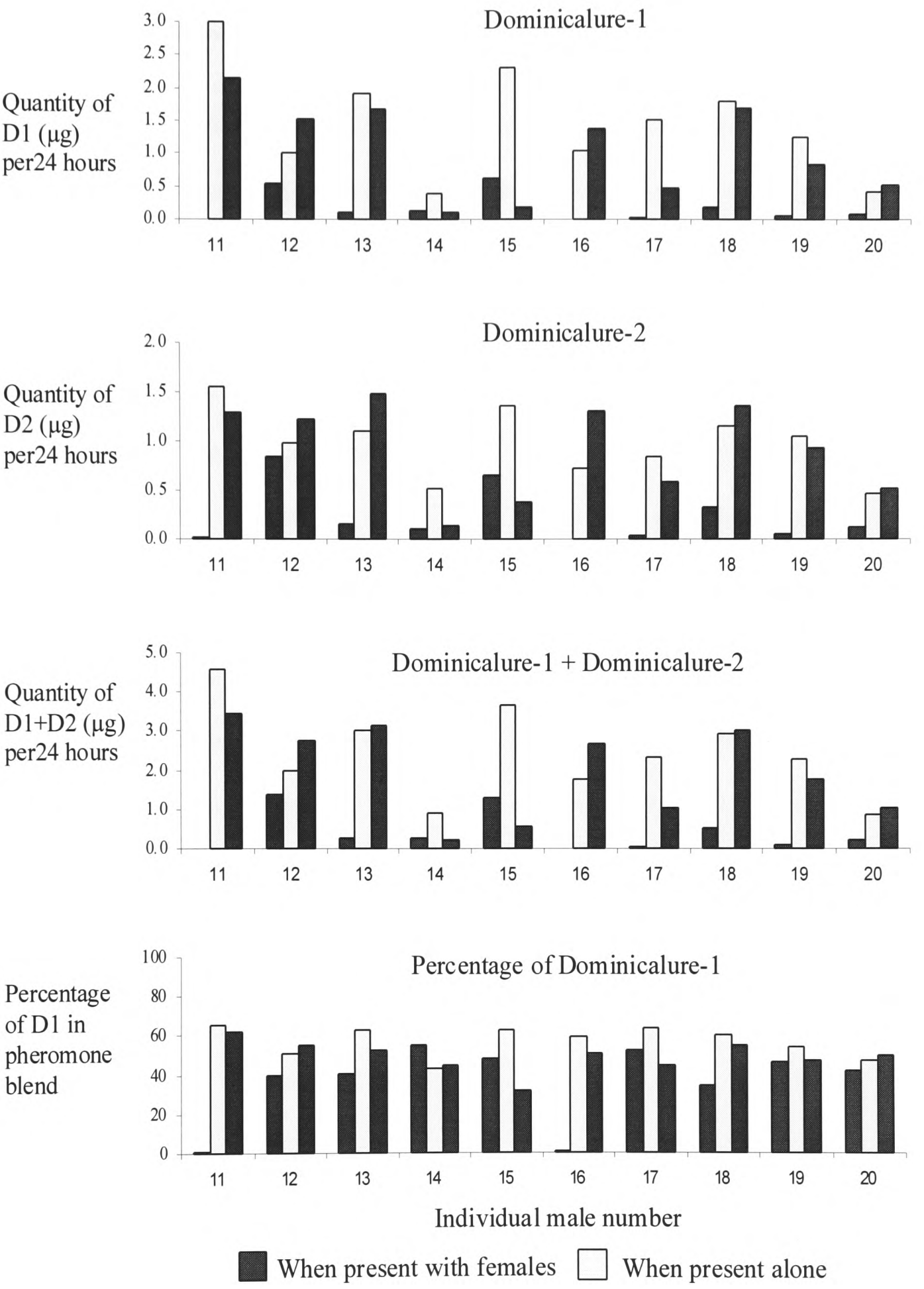


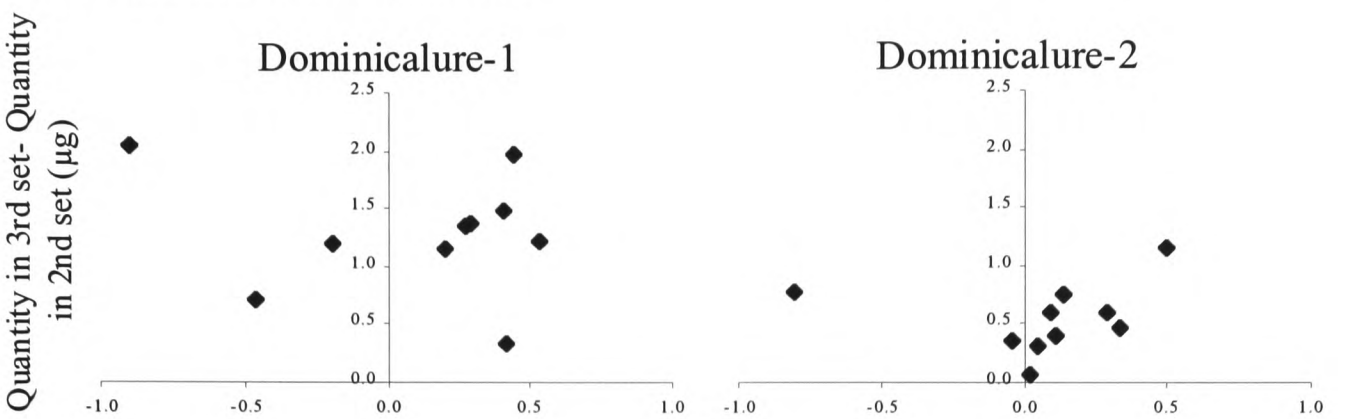
Figure 7.4b: Quantity (µg) of the pheromone components Dominicalure-1, Dominicalure-2, total quantity of both components, and percentage of Dominicalure-1 in the pheromone blend produced by individual *R. dominica* males when present alone or with seven females in twenty four hours; test was initiated with a male present with seven females

- *Is the magnitude of change/modification in pheromone signalling by males due to the presence or absence of females consistent?*

To determine whether males are consistent in their sensitivity to the presence or absence of females, the differences in the quantity of the pheromone components D1 and D2 (for the same beetle) between 1st & 2nd and 2nd & 3rd entrainments were plotted against each other. To calculate the difference between the sets, the quantity of pheromone produced in the case of males present with females was subtracted from that in the case of lone males. No strong correlation was apparent (Figure 7.5) which suggests that there was no regular change in the pheromone output between sets. However, as most values in the plots were positive it suggests that pheromone output in set 2 tended to be different from set 1 or set 3.

There was a significant positive correlation between the amounts of pheromone components D1 and D2 released by both lone males ($r = 0.91$, $p<0.001$, $n = 30$) and males with females ($r = 0.95$, $p<0.001$, $n = 30$) (Figure 7.6).

a. Test initiated with a lone male



b. Test initiated with a male with seven females

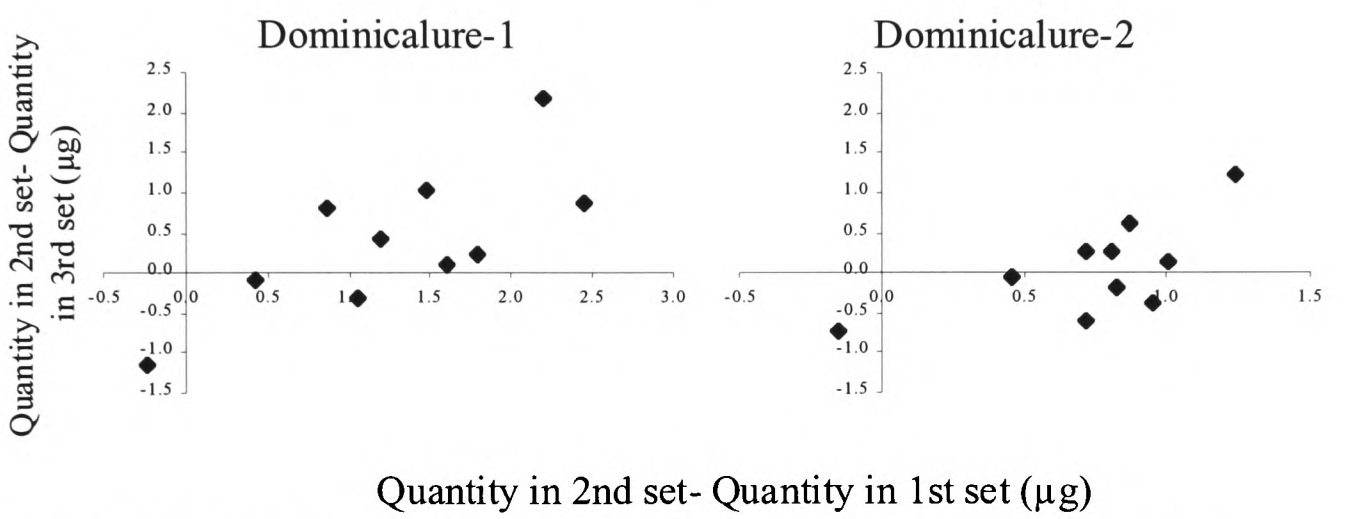


Figure 7.5: Correlation between the difference in the quantity of pheromone produced by the same males in first and second sets of entrainments and the difference in the quantity of pheromone produced by the same beetle in second and third set of entrainments

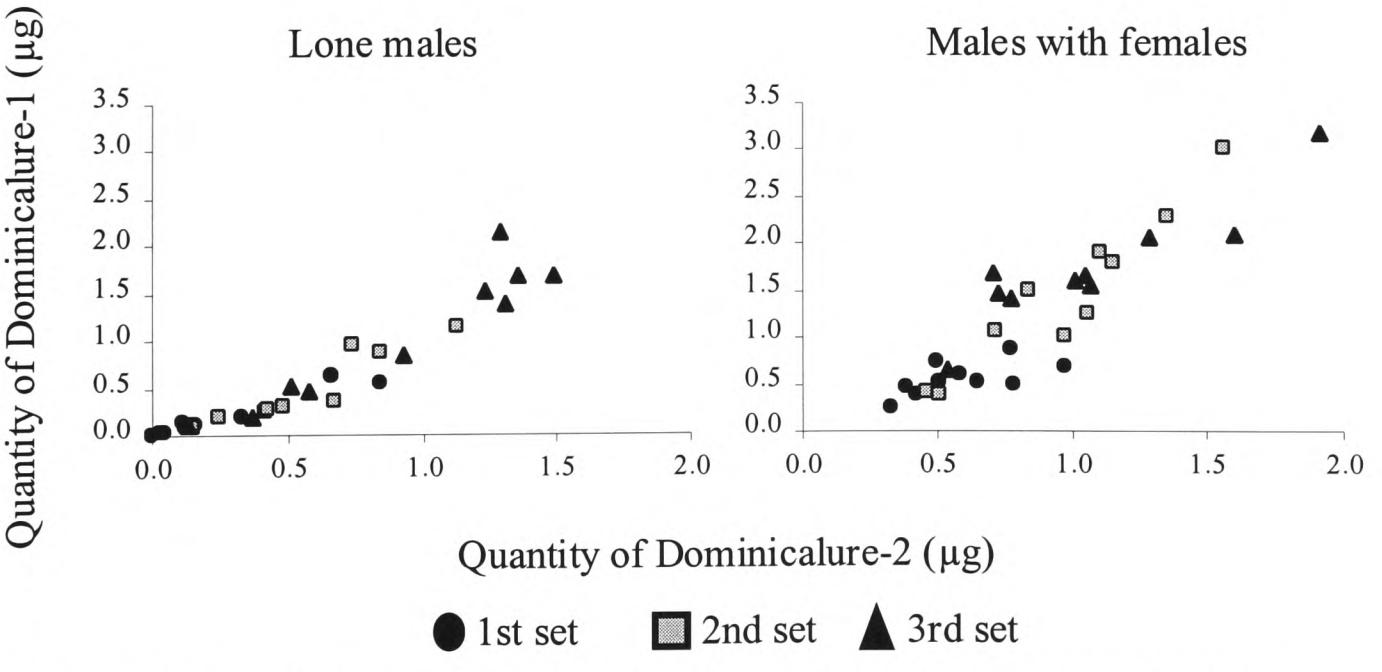


Figure 7.6: Correlation between the quantities of the pheromone components Dominicalure-1 and Dominicalure-2 produced in 24 hours by lone males and males with seven females

Olfactometer test

- *Is there any difference in the behavioural response of the beetles to pheromone signals from a lone male or a male with females?*

In all three sets, adult *R. dominica* spent more time in the zone receiving volatiles from a lone male than the zone receiving volatiles from a male with seven females or clean air (controls) (Figure 7.7). There was significant heterogeneity among treatments for males (1st set: Chi-Sq.=19.69, df = 3, $p<0.001$, 2nd set: Chi-Sq.=20.50, df = 3, $p<0.001$, 3rd set: Chi-Sq.=22.74, df = 3, $p<0.001$) and females (1st set: Chi-Sq.=15.35, df = 3, $p<0.01$, 2nd set: Chi-Sq.=38.30, df =3, $p<0.001$, 3rd set: Chi-Sq.=34.33, df = 3, $p<0.001$).

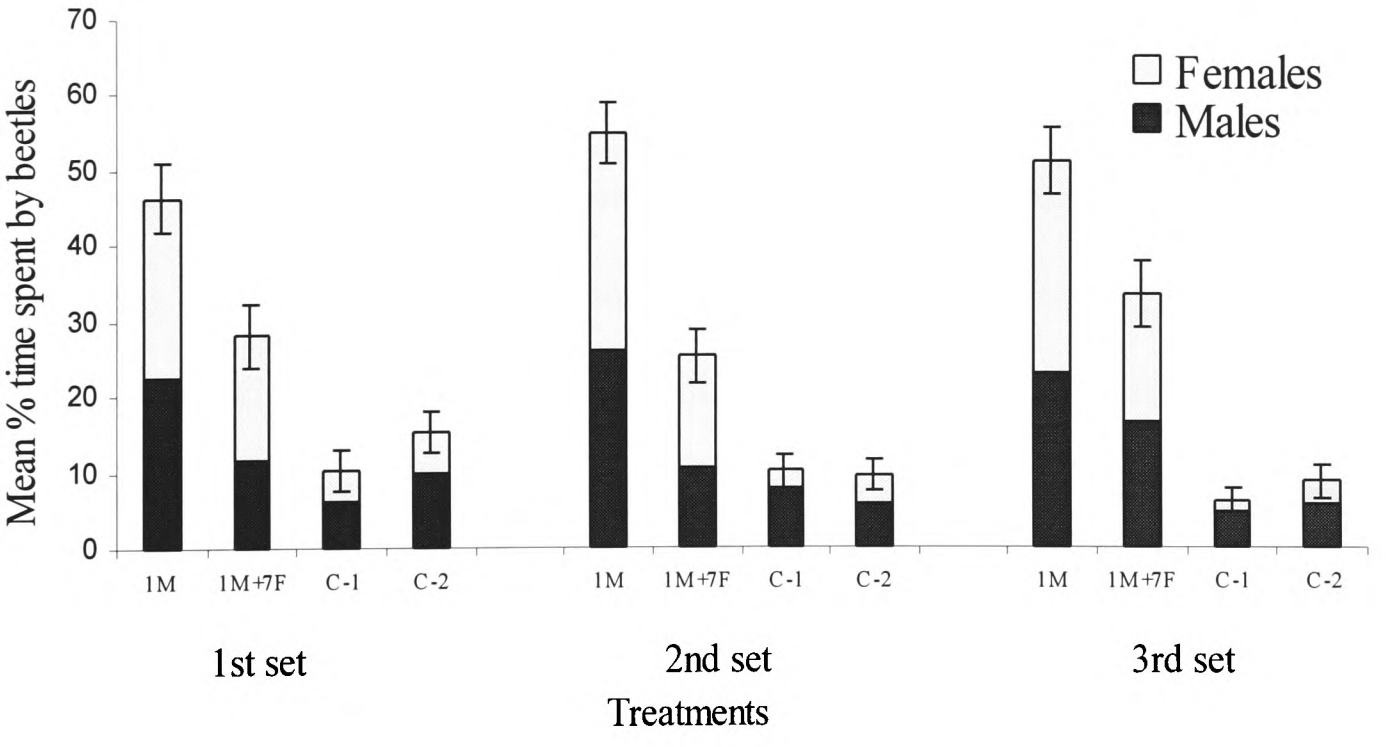


Figure 7.7: Mean percentage of time (5 minutes) spent by each adult *R. dominica* in different odour zones of a four-choice airflow olfactometer (standard error for males and females combined); there were ten replications of each treatment (source of odour/volatiles) in each set and responses of four males and four females were observed for every one pair of treatments
NB. M = Male; F= Females; C = Control

Subsequent pairwise analysis of the data between different treatments for each of the three sets showed significant differences between most of the combinations for both males and females (Table-7.6). Analysis of male and female data pooled, was possible as there was no significant difference in response between males and females for male alone ($p=0.84$, $p=0.64$ and 0.23 for 1st, 2nd and 3rd set, respectively), 1male+7females ($p=0.28$, 0.48 and 0.72 for 1st, 2nd and 3rd set, respectively), control-1 ($p=0.82$, $p=0.17$ and $p=0.20$ for 1st, 2nd and 3rd set, respectively) and control-2 ($p=0.09$, $p=0.47$ & $p=0.11$ for 1st, 2nd and 3rd set, respectively). As expected the pooled data showed significant heterogeneity among treatments for first (Chi-Sq.=37.08, df = 3, $p<0.001$) second (Chi-Sq.=66.42, df = 3, $p<0.001$) and third set (Chi-Sq.=67.02, df = 3, $p<0.001$), and subsequent pairwise comparisons between treatments showed significant difference between all the treatment combinations except between control-1 and control-2 (Table-7.6).

When the total numbers of beetles (males and females from three sets combined) preferring each of the four zones of the olfactometer test arena were compared, there was clearly a stronger response to volatiles from lone males (Table-7.7). The same is suggested by the percentage of time spent by beetles in the different zones.

Table-7.6: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from *R. dominica* males present alone or with seven females, or clean air (control); data were analysed using Wilcoxon test for two-related samples

	Treatment combinations	Males		Females		Pooled	
		Z	p-value	Z	p-value	Z	p-value
1st set	Male alone & 1male+7females	-2.18	0.03	-1.08	0.28	-2.08	0.04
	Male alone & Control-1	-3.20	<0.001	-3.99	<0.001	-5.13	<0.001
	Male alone & Control-2	-2.74	<0.01	-3.76	<0.001	-4.45	<0.001
	1male+7females & Control-1	-1.68	0.09	-3.03	<0.01	-3.26	<0.001
	1male+7females & Control-2	-0.06	0.95	-2.92	<0.01	-2.01	0.04
	Control-1 & Control-2	-1.47	0.14	-0.64	0.52	-1.65	0.10
2nd set	Male alone & 1male+7females	-3.02	<0.01	-2.08	0.04	-3.71	<0.001
	Male alone & Control-1	-3.41	<0.001	-5.00	<0.001	-6.02	<0.001
	Male alone & Control-2	-4.05	<0.001	-4.51	<0.001	-6.16	<0.001
	1male+7females & Control-1	-1.27	0.21	-3.44	<0.001	-3.43	<0.001
	1male+7females & Control-2	-2.07	0.04	-2.97	<0.01	-3.61	<0.001
	Control-1 & Control-2	-0.37	0.71	-0.60	0.55	-0.08	0.93
3rd set	Male alone & 1male+7females	-1.19	0.23	-1.87	0.06	-2.13	0.03
	Male alone & Control-1	-4.04	<0.001	-4.96	<0.001	-6.42	<0.001
	Male alone & Control-2	-4.04	<0.001	-4.53	<0.001	-6.04	<0.001
	1male+7females& -Control-1	-3.40	<0.001	-3.61	<0.001	-4.98	<0.001
	1male+7females & Control-2	-2.94	<0.01	-3.35	<0.001	-4.46	<0.001
	Control-1 & Control-2	-0.31	0.76	-1.12	0.26	-0.90	0.37

Table-7.7: Total number of beetles (male + female) in all three sets combined, favouring different zones of test arena in the four choice airflow olfactometer and percentage of the time spent by each beetle in those zones

	Number of beetles	Mean time spent
Lone males	140	51.06%
Male with 7 females	70	29.31%
Control-1	15	9.55%
Control-2	15	10.07%

- *Is there any difference between the responses of males and females?*

a. Do male and female responders show the same level of response to odour sources?

The response of males or females to both the odour sources (i.e. male signallers present alone and male signallers present with females) combined, showed that the females show greater response to the odour source than males. The female response was significantly greater than male response for the second ($z = -2.31$, $p = 0.02$, $n = 40$) and third ($z = -2.03$, $p=0.04$, $n=40$) set, but the difference was not significant for the first set.

b. Do male and female responders differ in their response to the same odour source?

The females showed slightly greater response than males to volatiles from males present alone. On average, each female spent 48%, 58% and 56% of its time in the zone receiving volatiles from lone males in first, second and third set, respectively, compared to 45%, 52% and 47% by males in first, second and third set, respectively. However, the differences between the responses of males and females were not statistically significant.

c. Do male and female responders show the same level of discrimination between pheromone signals?

The females showed higher level of discrimination between the male signallers present alone or with females (90%, 78% & 81%, respectively) than males (74%,

77% & 69%, respectively) in the first, second and third set, respectively. The difference was not significant for the second and third set, however, it was significant for first set ($z = -1.93$, $p = 0.05$, $n = 40$).

- *Which characteristics of the pheromone signal are most correlated to response?*

In order to investigate which pheromone characteristic(s) might be responsible for the observed responses in bioassay, comparisons were made between the chemical data and the behavioural data available for each pair of male signallers. The preference of the responding beetles given the choice of two signals was calculated using the formula below.

Response to lone male (% time spent in the zone)	-	Response to male with females (% time spent in the zone)

The average of the percentage response of four beetles tested for the same male signaller was calculated (separately for males and females) and plotted against different possible factors effecting the response of the beetles towards a volatile source. These different factors were:

D1 released by lone male	-	D1 released by male with females
D2 released by lone male	-	D2 released by male with females
D1-D2 released by lone male	-	D1-D2 released by male with females
% D1 in the blend released by lone male	-	% D1 in the blend released by males with females

There was no strong correlation between any of these factors and response of the beetles. An example of the data is given in Figure 7.8.

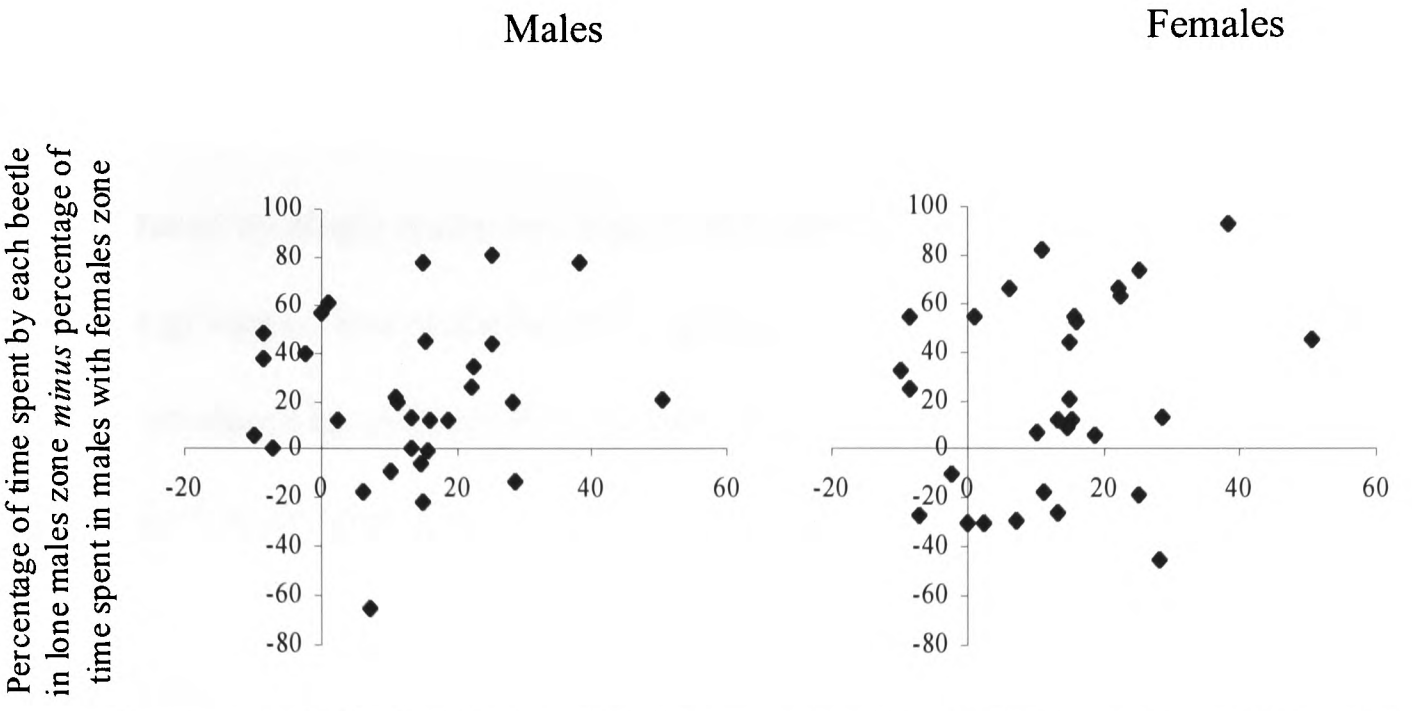


Figure 7.8: Correlation between the preference of male or female beetles for the zones of a test arena receiving volatiles from lone males or males present with seven females and the difference in %D1 in the pheromone blends released by these beetles

7.3.3. Effect of the presence of conspecific males on the pheromone signalling by *R. dominica* males

Mean total quantities of the pheromone and both of its components D1 and D2 produced per male were greater in single males than any of the groups of males (Figure 7.9). The quantities of pheromone per male decreased as the number of the beetles increased up to the four males and then they increased slightly in the case of six or eight males. This was true for both pheromone components.

The quantities of pheromone components (D1 and D2) and pheromone blend (D1+D2) produced per male in different treatments were significantly different from each other (Table-7.9). Pairwise comparison between the different treatments (Table-7.10) showed that total quantities of pheromone and the component D2

produced per male in the case of single males were significantly greater than those produced by individuals in the groups of four, six, or eight males. The quantity of D1 produced by single males was significantly greater than produced by individual males in groups of four or six but not in groups of eight. The quantities of D2 and D1+D2 produced by individuals in groups of two were significantly greater than four or six but not than eight.

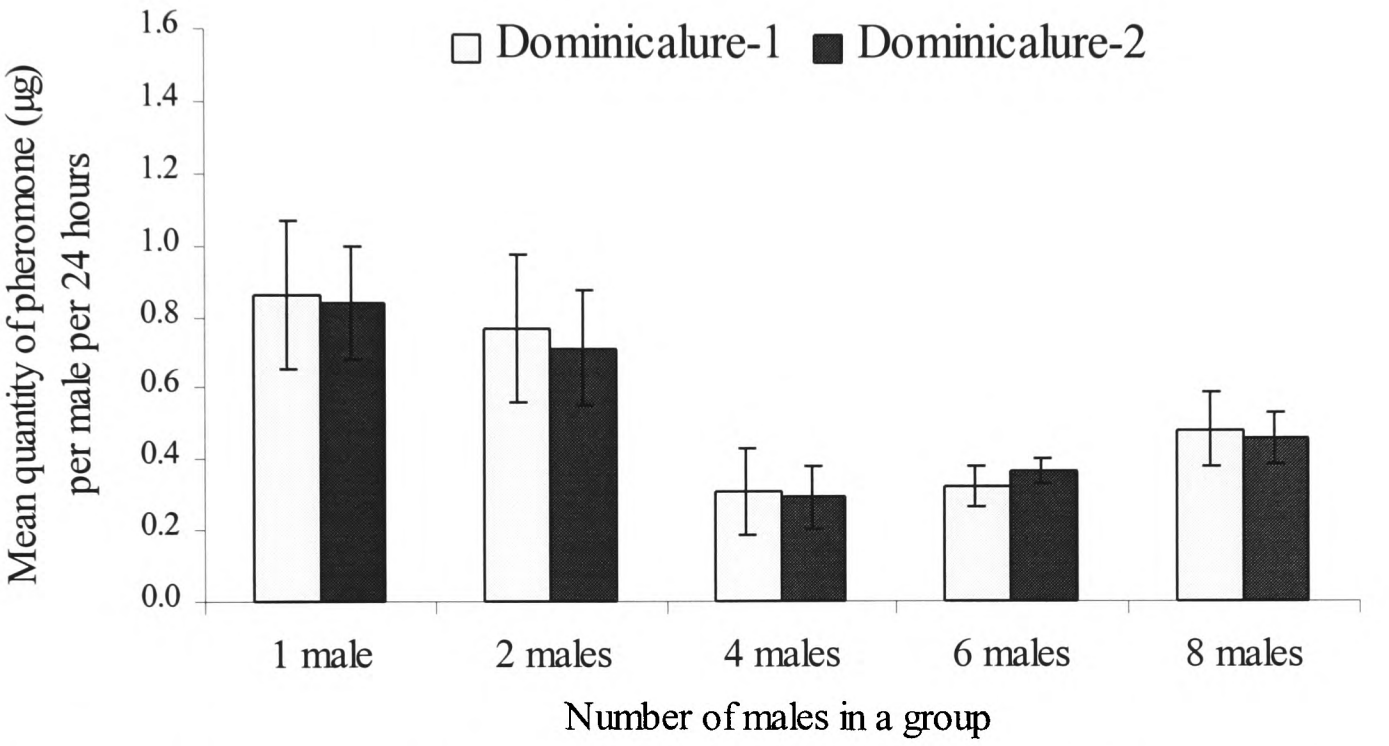


Figure 7.9: Mean quantities of the pheromone components Dominicalure-1 and Dominicalure-2 produced per male during twenty four hours by different sized groups of *R. dominica* males; error bars represent standard error (n=12)

Table-7.9: One-way analysis of variance for quantities of Dominicalure-1 (D1), Dominicalure-2 (D2), total quantity (D1+D2), and percentage of D1 in the blend produced per male by different groups of *R. dominica* males (n=12)

Variable	D.F.	Sum of Sq.	Mean Sq.	F-Ratio	F-Prob.
D1	4	2.99	0.75	2.77	0.04
D2	4	2.51	0.63	4.12	<0.01
D1+D2	4	10.90	2.73	3.59	<0.01
%D1	4	70.82	17.70	0.18	0.95

When percentage of the D1 in the pheromone blend was calculated it was similar in all the treatments (Figure 7.10) and there was no statistical evidence of any difference (Table-7.9).

Table-7.10. Pairwise comparison between different treatments for the total quantity of the pheromone blend (D1+D2), and its two components Dominicalure-1 (D1) and Dominicalure-2 (D2), using Least Significant Difference test (LSD) ('*' indicates significant difference and '+' indicates non-significant difference)

Treatments	Single male			Two males			Four males			Six males		
	D1	D2	D1+D2	D1	D2	D1+D2	D1	D2	D1+D2	D1	D2	D1+D2
Two males	+	+	+									
Four males	*	*	*	*	+	*						
Six males	*	*	*	*	+	*	+	+	+			
Eight males	*	+	+	+	+	+	+	+	+	+	+	+

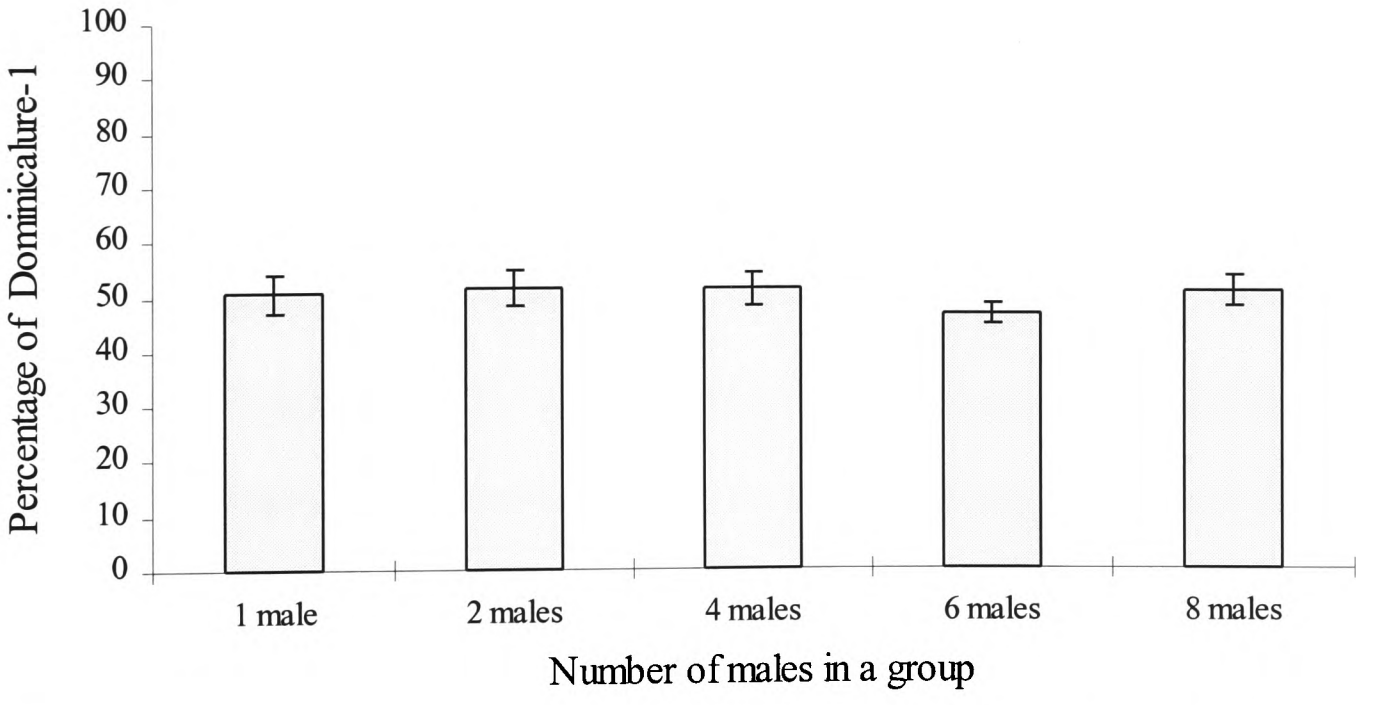


Figure 7.10: Percentage of the pheromone component Dominicalure-1 in the pheromone blend produced by *R. dominica* males in groups comprising of different numbers; error bars represent standard error (n=12)

7.4. DISCUSSION

These studies have shown that *R. dominica* males produced smaller quantities of pheromone when present with females than when present alone. The quantity of the pheromone component D1 was considerably reduced when single males were confined with different numbers of females (section 7.3.1), but the quantity of D2 was not affected much, thus changing the ratio of D1 to D2. In the more detailed comparison between a lone male and male with seven females (section 7.3.2), the release rate of both the pheromone components were reduced, however, as in earlier case, D1 showed greater sensitivity to the presence of females than D2. This reflected in the change of ratio of D1 to D2. Mayhew and Phillips (1994) did not observe any significant difference in the level of the pheromone production between males of *R. dominica* present alone or paired with females. The difference in the results of Mayhew and Phillips (1994) and the present studies may be due to the reasons that they (1) did not have enough replications, collecting data from only four mated males, (2) did not consider difference in the ratio of the pheromone components between mated and unmated males, and (3) pairing with one female may in any case not cause a sufficient change for a significant result in a test with a low degree of replications. The actual number of females may be important as Vite *et al.* (1972) found that pheromone production by *Ips calligraphus* (Germar) males ceased only when 3 or more females were present.

Study of the pheromone output by individual males showed that the change in the pheromone signals of males caused by the presence of females is reversible (Figure 7.4a & 7.4b). However, there was no regular pattern in the magnitude of

the change in the pheromone signals when males were confined and separated from the females.

The change in the pheromone signals of males due to the presence of females raises the question, what significance does this modification in signal have for the receiving conspecific individuals? Is this message intended for both conspecific males and females? The olfactometer test was designed to address these questions. The results showed that in all three sets of olfactometer test, the beetles clearly preferred the pheromone signals released by the lone males to that released by the males with females. In bark beetles, the reduced production and/or release of pheromone by males after mating appears to play a major function in the process of terminating the aggregation phase of host colonisation (Byers, 1981). Response of the *R. dominica* was not statistically correlated to the different characteristics of the pheromone signals but study of each pair of pheromone signals and responding beetles individually revealed that in most of the cases beetles were attracted to the pheromone signals with a higher percentage of D1.

There was no statistically significant difference between the responses of males and females but the females showed slightly greater attraction for the pheromone signals both from the lone males as well as from the males with females. Similar differences between the responses of males and females were observed by Hodges *et al.* (1998) and Scholz *et al.* (1997a) in the field experiments for the related species *Prostephanus truncatus*.

Males present in groups produced smaller quantities of pheromone (per male) than single males. These results are in agreement with the findings of Mayhew and

Phillips (1994) who reported a decrease in pheromone production per male by *R. dominica* males in groups of 5 or 15. Dowdy *et al.* (1993) also obtained similar results using cultures of unsexed adults of different densities. Similar results have also been reported for several other insects such as *Anastrepha suspensa* (Loew) (Nation, 1990), *Tribolium castaneum* (Herbst) (Hussain, 1993), *Carpophilus antiquus* Melsheimer (Bartelt *et al.*, 1993), *Carpophilus davidsoni* Dobson (Bartelt and James, 1994), *Carpophilus obsoletus* Erichson (Petroski *at el.*, 1994). In the present case, when groups of males are producing a smaller quantity of pheromone per male, one cannot be sure whether every individual in a group is producing less, or a few are producing the 'normal' quantity and a few are not producing at all. If every individual in the group is producing less, it can be a strategy by individual males to conserve energy from pheromone biosynthesis (Mayhew and Philips, 1994), as pheromone synthesis is an energy consuming process (Schlyter and Birgersson, 1989). Unlike the presence of females, which showed a marked effect on the ratio of the pheromone components produced by *R. dominica* males, the presence of conspecific males apparently did not show any effect on the ratio of D1 to D2 in the pheromone blend.

As in the previous chapter, generally, the differences in the pheromone released by males present alone or with females detected by capillary gas chromatography analysis were successfully demonstrated by the olfactometer tests. Chromatography analysis showed greater quantities of pheromone, especially pheromone component D1, were produced by lone males. In olfactometer test, beetles showed a greater response to the volatiles from lone males. However, when each pair of source and responding beetles were compared individually, the

magnitude of the difference between the response of the beetles to volatiles from lone males and males with females did not correlate with the magnitude of the difference in the pheromone between males present alone and with females.

In conclusion it can be suggested that *R. dominica* males in the presence of conspecifics produce smaller quantities of their aggregation pheromone. Pheromone signals produced in the presence of females have a lower percentage of the pheromone component Dominicalure-1 but this change in the ratio of the pheromone components is not affected in the presence of other males. Further, the pheromone signals produced by the males in the presence of females make them less attractive for the conspecific individuals receiving these signals.

There are still many questions to be answered. For example, what is the reason for the observed modification in pheromone signals in presence of females, is it a response to mating or physical presence of females? Which characteristic(s) in the pheromone signal of a male make it more or less attractive? What is the real reason for reduced pheromone production in groups of males? Further studies are needed to provide answers of these questions.

Chapter:8

Which characteristics of a pheromone signal affect the beetle's response?

8.1. INTRODUCTION

It was demonstrated in the previous chapters that external factors such as host-type or presence of females could affect pheromone signalling by *R. dominica* males (see Chapter 6 & 7). It was also shown that these modified pheromone signals are less attractive to responding conspecifics than 'normal' signals. However, the attempt to determine which specific characteristics of a pheromone signal are the most correlated with the observed responses was not successful. Therefore, it was decided to investigate the response of the beetles to the individual components of pheromone and blends with widely different ratios of the two components. In order to prepare such blends synthetic pheromone had to be used. The use of synthetic pheromones is very helpful for experimental studies of insect behaviour as different ratios in the pheromone blend and individual components can be tested, which is not possible when using natural pheromone sources (i.e. living insects). Many scientists have used synthetic pheromones to study various aspects of insect-pheromone relationship (Burns and Teal, 1989; Mayer and McLaughlin, 1991; Quartey and Coaker, 1993; Valeur and Lofstedt, 1996; Shetty and Hough-Goldstein, 1998; Zhang *et al.*, 1998).

Cogburn *et al.* (1984) reported that pheromone components D1 and D2 individually and in different ratios were equally attractive to *R. dominica* adults in field traps. However, no studies have been made to compare the response of *R. dominica* adults in short-range bioassays to blends with different ratios and to D1 or D2 individually. This becomes more important in the light of the results that have been reported for the related species *Prostephanus truncatus*. The adults of *Prostephanus truncatus* are more attracted to Trunc-call 2, the major component of their aggregation pheromone, than to Trunc-call 1 (Leos-Martinez *et al.*, 1995). However, when the concentration of Trunc-call 2 is high, then a mixture with Trunc-call 1 becomes significantly more attractive than Trunc-call 2 alone (Hodges *et al.*, 1998).

Before undertaking the experiments with synthetic pheromone, it was imperative to establish that the synthetic pheromone lures to be used in these studies, are as attractive as the natural pheromone (i.e. adult males). To do this, an experiment was undertaken to compare the response of adult *R. dominica* males and females to natural and synthetic pheromone sources. To make a valid comparison it was important to ensure that the quantities of pheromone released from the synthetic pheromone sources were more or less similar to those released by males. In some storage insect pests, production of pheromone differs according to time of day (Hammack *at el.,* 1976; Abdel-Kader *et al.*, 1987), however, the behaviour of *R. dominica* in this regard is unknown. Therefore, the pheromone production by *R. dominica* males was quantified at different times of day. This information helped to decide the most appropriate time for the olfactometer tests regarding comparison of natural and synthetic pheromone sources.

8.2. METHODS AND MATERIALS

Insects

R. dominica adults were removed from culture (see section 2.2), sexed (see section 2.3) and kept singly on kibbled wheat grain for three days. The males were then moved into maize grains (one male per grain), already prepared by drilling approximately 4 mm deep holes in them with a 1.5 mm drill. The beetles were allowed to feed in the grains for seven days before being used in the experiments as pheromone source and/or their pheromone output was measured.

Beetles used to observe responses in olfactometer tests were sexed and kept singly on kibbled wheat grain for seven days before they were tested.

Source of synthetic pheromone

Polythene vials (outer diameter = 8 mm, height = 26 mm, volume = 0.5 ml) (Just Plastic, UK) loaded with different quantities of synthetic Dominicalure-1 and/or Dominicalure-2, were provided by the Chemical Ecology Group, Pest Management Department, Natural Resources Institute and stored in a sealed aluminium foil bag at -20°C before use. The synthetic pheromone used was about 97% pure and isomerically the same as released by *R. dominica* males.

Pheromone entrainment

The details and arrangement of pheromone entrainment equipment are presented in section 2.5.

Olfactometer test

The details and arrangement of olfactometer equipment are presented in section 2.4.

Statistical analysis

See section 2.6 for statistical methods used for analysis of data.

8.2.1. Pheromone production by *R. dominica* males at different times of day

Experimental procedure

The male beetles present in maize grains were kept separately for seven days but during pheromone entrainments there were four males in the same entrainment chamber, but in the separate grains. Three entrainment chambers were connected to one Porapak Q filter, so that pheromone from twelve males was collected on the same filter. The collections were made for whole light period i.e. 08.00 to 20.00 hours (British Standard Time) consisting of six two-hour periods i.e. for 08.00h-10.00h, 10.00h-12.00h, 12.00h-14.00h, 14.00h-16.00h, 16.00h-18.00h and 18.00h-20.00h periods. The whole procedure was repeated on a further seven consecutive days, making eight replications for each of the two-hour sampling periods.

In the same arrangements as described above (three entrainment chambers, each with four males in these, connected to one Porapak Q filter), pheromone was collected from the same beetles for continuous periods of twelve and twenty four-hours also (i.e. for 20.00h-08.00h & 08.00h-08.00h). This was also replicated eight times.

Statistical analysis

A paired-samples t-test was used to make pairwise comparisons between quantities of pheromone produced at different times of day.

8.2.2. Preference of *R. dominica* adults for natural or synthetic pheromone sources

Experimental procedure

A group of four *R. dominica* males each present in a separate maize grain was used as a natural pheromone source. A polythene vial loaded with 0.16mg of Dominicalure-1 and 0.12mg Dominicalure-2 was used as the synthetic pheromone source. Four maize grains drilled as stated above were placed with the synthetic pheromone-loaded polythene vial to ensure that the volatiles emitted from both the sources were as similar as possible. Each pheromone source had eight replicates.

The male beetles present in maize grains were kept separately for seven days. However, when they were used as pheromone sources in olfactometer tests, four males each in a separate grain, were placed in the same flask. In this way, pheromone released by a group of four males was tested against the synthetic pheromone released from the polythene vials. The polythene vials loaded with synthetic pheromone were prepared fifteen days before they were used in the experiment. The vials were taken out of the packing, left in a fume cupboard at room temperature (25±5°C) for three hours before they were used in the olfactometer as pheromone sources.

Pheromone entrainments from one half (four replications) of either of the pheromone sources were made before, and from the other half after, the olfactometer tests were performed. Entrainments from synthetic pheromone-loaded polythene vials were made for one-hour periods while entrainments from groups of males were made for six-hour periods from 10.00h to 16.00h. During entrainments, four males each in a separate maize grain were present in the same entrainment chamber so that the pheromone released by these four males could be collected on one filter.

The response of five males and five females was tested for every one pair of natural and synthetic pheromone sources. In this way, responses of a total of forty males and forty females were recorded.

8.2.3. Which characteristics of a pheromone signal affect the beetle response?

Experimental procedure

Investigations of beetle response to different combinations of pheromone outputs were planned for this test (Table-8.1). A number of preliminary entrainments were made from vials loaded with different quantities of the synthetic pheromone to decide which loadings would give the required pheromone outputs but unfortunately this was not achieved (see below).

The response of five males and five females was tested for each pair of pheromone sources, in this way the response of a total of forty males and forty females was recorded for each combination. The polythene vials loaded with synthetic pheromone were prepared fifteen days before they were used in the experiments.

The vials were taken out of the packing, left in a fume cupboard at room temperature (25±5°C) for three hours before they were used in the olfactometer as pheromone sources. Pheromone entrainments from one half (four replications) of either of the pheromone sources were made before, and from the other half after, the olfactometer tests were performed.

Table-8.1: The synthetic pheromone blends to be compared by beetle response and the polythene vial loadings required to achieve these blends

Dominicalure-1: Dominicalure-2			Dominicalure-1: Dominicalure-2	
Loading in vials	Expected output ratio		Expected output ratio	Loading in vials
0.24mg:0.12mg	1:1	*vs*	1:2	0.16mg:0.16mg
0.24mg:0.12mg	1:1	*vs*	2:1	0.30mg:0.10mg
0.24mg:0.12mg	1:1	*vs*	D1	0.45mg
0.24mg:0.12mg	1:1	*vs*	D2	0.20mg
0.45mg	D1	*vs*	D2	0.20mg

Unfortunately the tests on the pheromone entrainment vials showed that the ratios and quantities of the pheromone components in the output were not those that had been predicted. Therefore, the desired results were not achieved. It is possible that the desired quantities of D1 and D2 loaded into the vials had been reversed. Nevertheless, the results of these tests are being presented, to provide some insights into the beetle-pheromone relationship.

8.3. RESULTS

8.3.1. Pheromone production by *R. dominica* males at different times of day

- *Is there any difference in the pheromone production by R. dominica males at different times of day?*

Mean total quantities of the pheromone components Dominicalure-1 & Dominicalure-2 released by *R. dominica* males in different two-hour periods between 08.00 to 20.00h are presented in Figure 8.1. The amount of pheromone released between 08.00h and 16.00h was similar for each two-hour period. However, there was a considerable increase in the pheromone release in both the two-hour periods between 16.00h and 20.00h. Percentage of D1 in the blend was similar in both the cases.

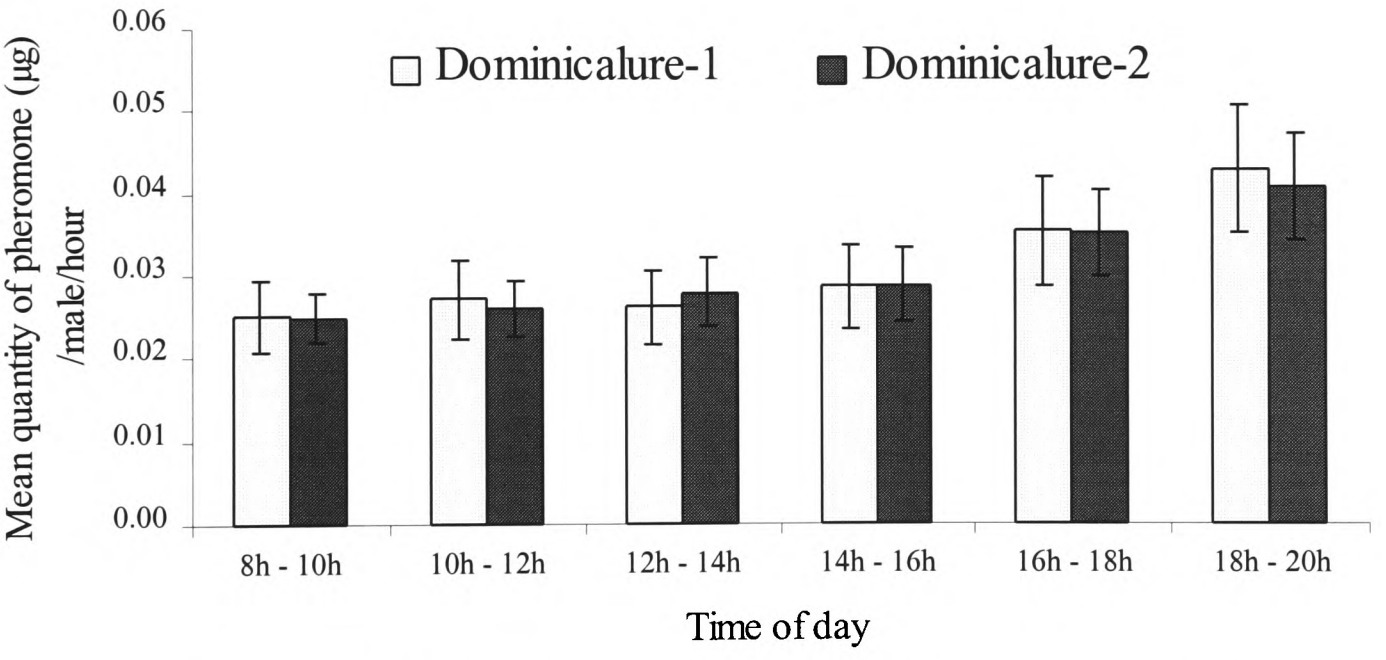


Figure 8.1: Mean quantities of pheromone components Dominicalure-1 and Dominicalure-2 released by twelve *R. dominica* males in two-hour periods at different times of day; quantities shown in the graph have been converted to per males per hour; error bars represent standard error (n=8)

Statistical analysis of the data showed no significant difference between the six two-hour periods for all the pheromone characteristics, D1, D2, D1+D2 and

percentage of D1 in the blend. However, as there was a considerable difference between the mean quantities of pheromone produced per male per hour in the morning (D1=0.025µg, D2=0.025µg) and evening (D1=0.043µg, D2=0.041µg), entrainment periods were analysed pairwise to check for statistically significant differences. This analysis showed that quantities of both the pheromone components D1 and D2 and total quantity (D1+D2) released in the periods of 16.00 to 18.00h or 18.00 to 20.00h were significantly greater than those released in any of the other periods (Table-8.2).

Table-8.2: Comparison between different treatments on one to one basis for the quantities of the pheromone components Dominicalure-1 and Dominicalure-2 produced by groups of twelve males at different times of day; P values given are calculated by using a paired sample t-test.

Treatments*	D1	D2	D1+D2	%D1
1st & 2nd	0.09	0.53	0.28	0.16
1st & 3rd	0.20	0.12	0.13	0.84
1st & 4th	0.01	0.13	0.04	0.67
1st & 5th	0.01	0.01	0.01	0.60
1st & 6th	<0.001	0.01	0.01	0.15
2nd & 3rd	0.57	0.31	0.72	0.26
2nd & 4th	0.16	0.13	0.11	0.77
2nd & 5th	0.01	0.01	0.01	0.04
2nd & 6th	0.01	0.01	0.01	0.41
3rd & 4th	0.05	0.68	0.30	0.43
3rd & 5th	0.01	0.01	0.01	0.51
3rd & 6th	0.01	0.01	0.01	0.05
4th & 5th	0.00	0.01	0.01	0.82
4th & 6th	0.01	0.01	0.01	0.30
5th & 6th	0.11	0.15	0.13	0.09

*1st =08.00-10.0h, 2nd = 10.00-12.00h, 3rd = 12.00-14.00h, 4th = 14.00-16.00h, 5th = 16.00-18.00h, 6th = 18.00-20.00h

- *Is there any difference in the pheromone production between day and night times?*

The mean quantity of pheromone produced per male per hour during the twelve-hour period of night/darkness from 20.00h to 08.00h was almost the same as that produced during the twenty-four hour period from 08.00h to 08.00h (Figure 8.2). Addition of all the values of two-hour periods of day/light from 0.800h to 20.00h gave a value which was little more than that produced during twenty four-hour period (08.00h to 08.00h) or twelve hour-period of night/darkness (20.00h to 0.800h). The ratio of the pheromone components D1 and D2 was similar for all the periods.

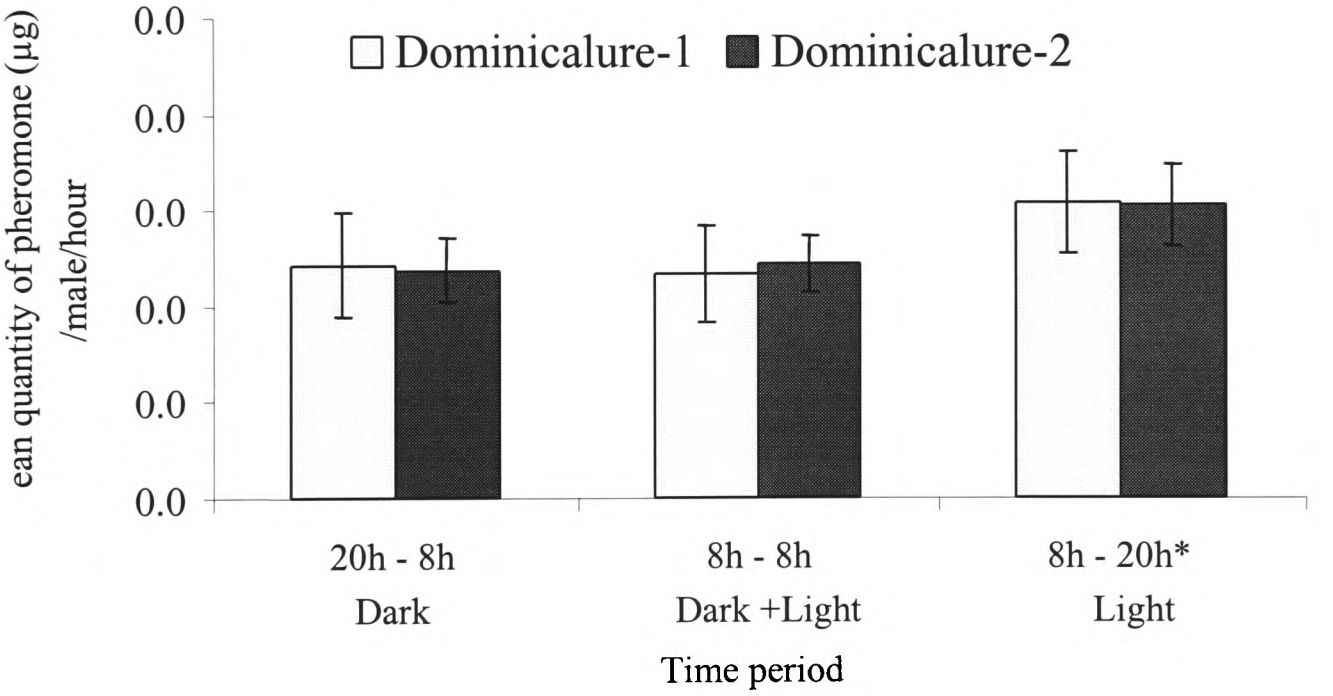


Figure 8.2: Mean quantities of pheromone components Dominicalure-1 and Dominicalure-2 produced by twelve *R. dominica* males during twelve hour-period of day/light, night/darkness and twenty four hours-period; quantities shown in the graph have been converted to per male per hour; error bars represent standard error (n=8).

* = Adding all the values for two-hour periods from 8.00h to 20.00h

8.3.2. Preference of *R. dominica* adults for natural or synthetic pheromone sources

Pheromone entrainments

Entrainments made from males and synthetic pheromone source showed that the quantity of D2 released in both cases was similar while the quantity of D1 was slightly greater for synthetic pheromone (Figure 8.3). Thus the total quantity of pheromone and percentage of D1 in the blend was 0.50µg and 62% for the synthetic pheromone source compared to 0.42µg and 56% for males. Statistical analysis showed that the quantity of D1 ($t = -2.53$, $df = 14$, $p = 0.02$) and total quantity of pheromone ($t = -2.17$, $df = 14$, $p = 0.048$) were significantly different while the quantity of D2 and percentage of D1 in the blend were not significantly different.

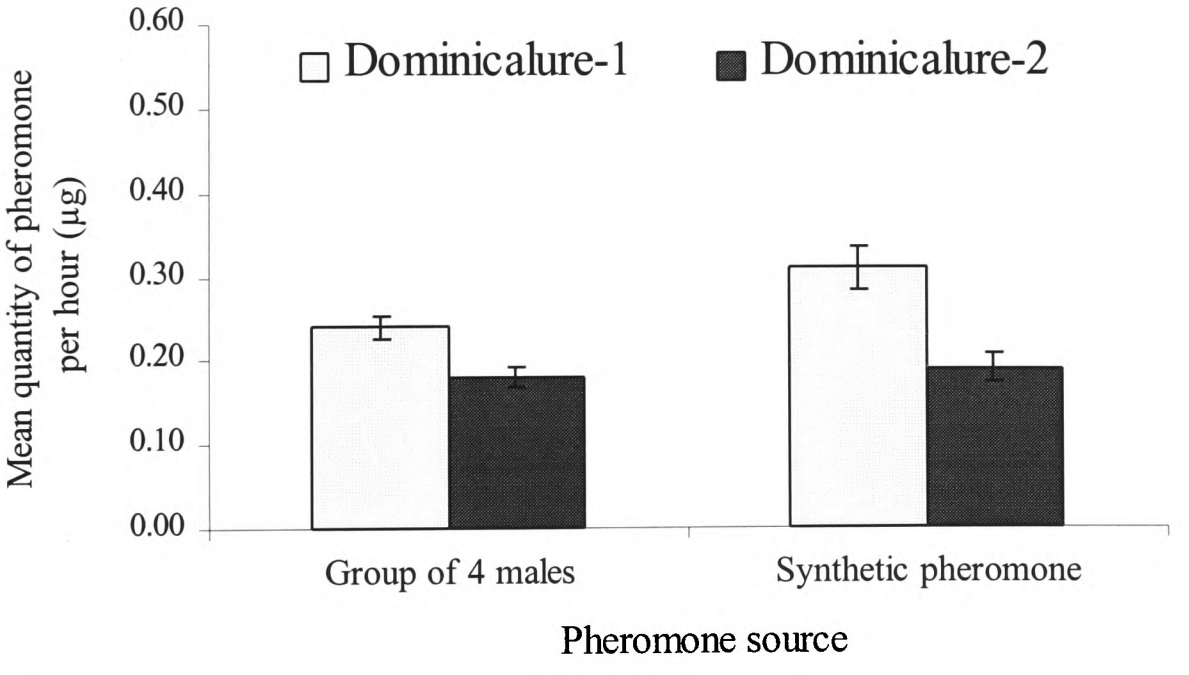


Figure 8.3: Mean quantity of pheromone released by groups of four males or synthetic pheromone source in one hour; error bars represent standard error (n = 8)

Olfactometer tests

Adult *R. dominica* spent similar times in the zones receiving volatiles from synthetic or natural pheromone source (Figure 8.4). There was significant heterogeneity among treatments for males (Chi-square=13.80, df=3, $p<0.01$), females (Chi-square=15.94, df=3, p=0.001), and data for males and females pooled (Chi-square=29.26, df=3, $p<0.001$). The analysis of the pooled data was possible as there was no significant difference between males and females for any of the treatments, natural pheromone (z=-0.47, p=0.64, n=40), synthetic pheromone (z=-0.02, p=0.99, n=40), control-1 (z=-0.65, p=0.52, n=40) or control-2 (z=-0.39, p=0.70, n=40).

Subsequent pairwise comparisons between different treatments for males, females and data for males and females pooled demonstrated statistically significant difference only between the pheromone sources and controls (Table-8.3).

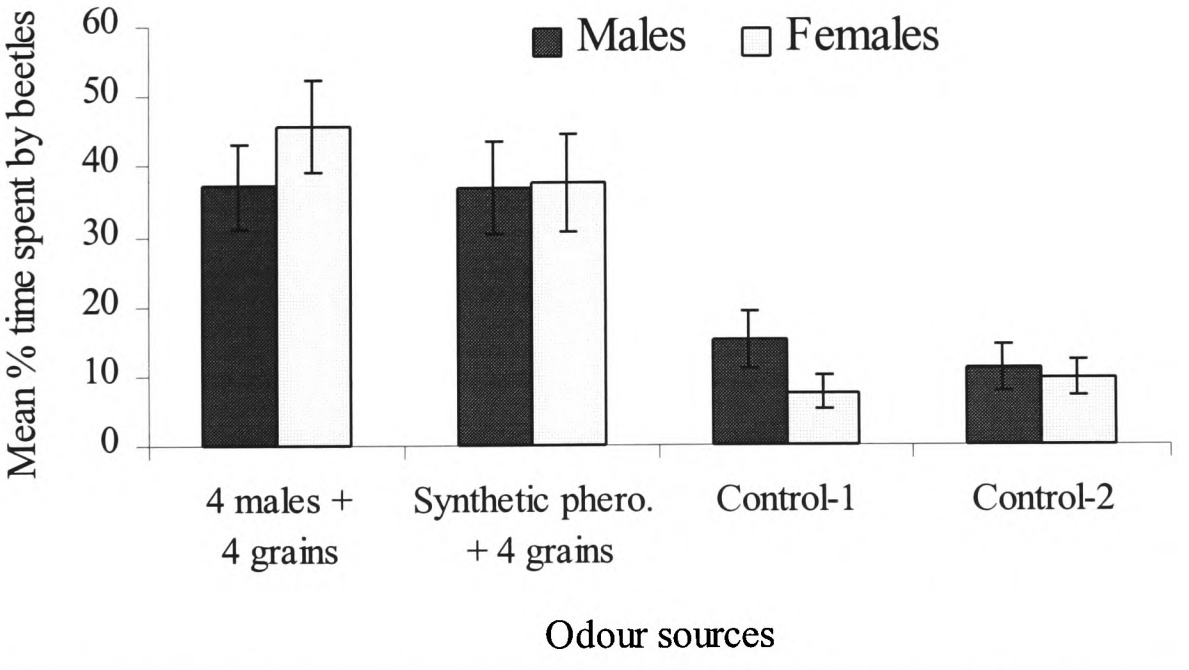


Figure 8.4: Mean percentage of time spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving volatiles from a group of four males, synthetic pheromone source or clean air; odour sources were replicated eight times and response of five males and five females was tested for each replication; error bars represent standard error (n=8)

Table-8.3: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from group of four males, synthetic pheromone source or clean air (control); data were analysed using Wilcoxon test for two-related samples (n=8)

Treatment comparisons	Males		Females		Pooled	
	Z	p-value	Z	p-value	Z	p-value
Four males *vs.* Synthetic pheromone	-0.08	0.94	-0.16	0.88	-0.15	0.88
Four males *vs.* Control-1	-2.67	<0.01	-3.94	<0.001	-4.83	<0.001
Four males *vs.* Control-2	-3.24	<0.001	-3.84	<0.001	-5.00	<0.001
Synthetic pheromone *vs.* Control-1	-2.31	0.02	-3.26	<0.001	-3.88	<0.001
Synthetic pheromone *vs.* Control-2	-2.83	<0.01	-2.77	<0.01	-3.93	<0.001
Control-1 *vs.* Control-2	-0.63	0.53	-0.74	0.46	-0.13	0.90

Is there any difference between males and females in the level of response or level of discrimination between odour sources?

The percentage of time spent by males or females in both the odour zones combined, indicated that females showed a slightly higher level of response (83%) than males (74%), however, the difference was not significant.

There was no significant difference between males (85%) and females (81%) in the level of discrimination between odour sources.

8.3.3. Which characteristics of a pheromone signal affect the beetle response?

Pheromone blend with 74% of D1* vs *pheromone blend with 59% of D1

Mean quantity of pheromone released from synthetic pheromone sources is shown in Figure 8.5 and response of males and females to these releases is presented in Figure 8.6. There was significant heterogeneity among treatments for males (Chi-

square=27.02, df=3, p<0.001) and females (Chi-square=38.60, df=3, p<0.001). However, subsequent pairwise comparisons between treatments demonstrated no significant difference between responses to the pheromone blend with 74% of D1 or to the blend with 59% of D1 for males or females (Table-8.4).

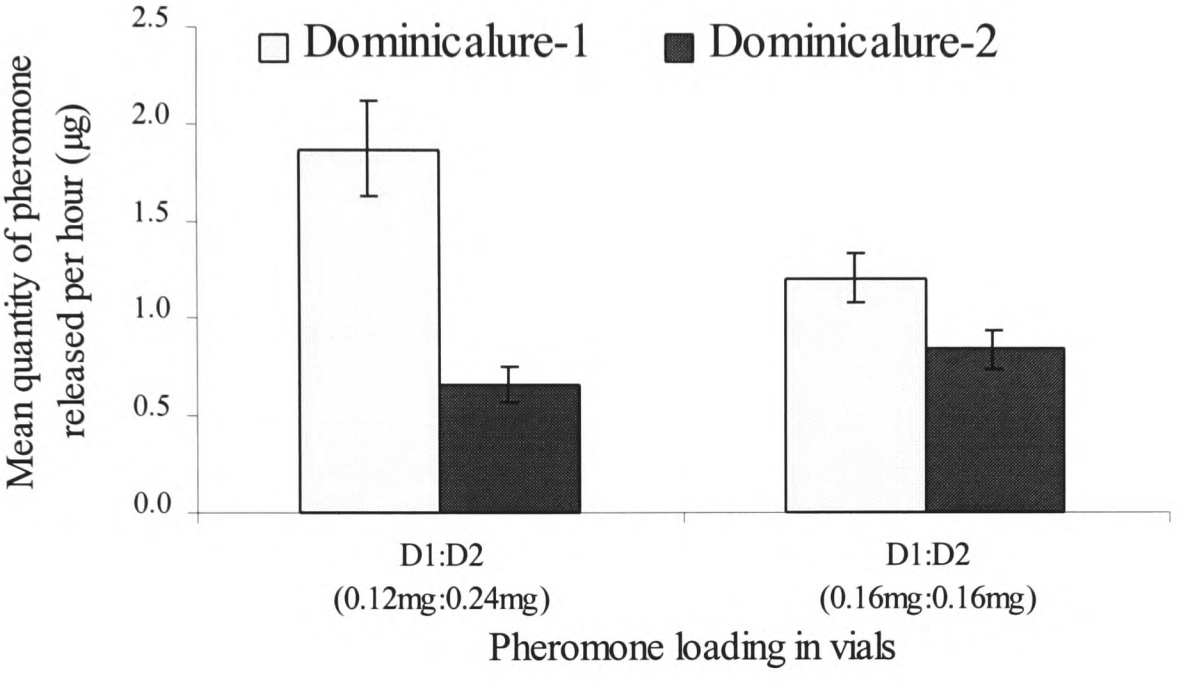


Figure 8.5: Mean quantity of pheromone released from synthetic pheromone sources in one hour; error bars represent standard error (n = 8)

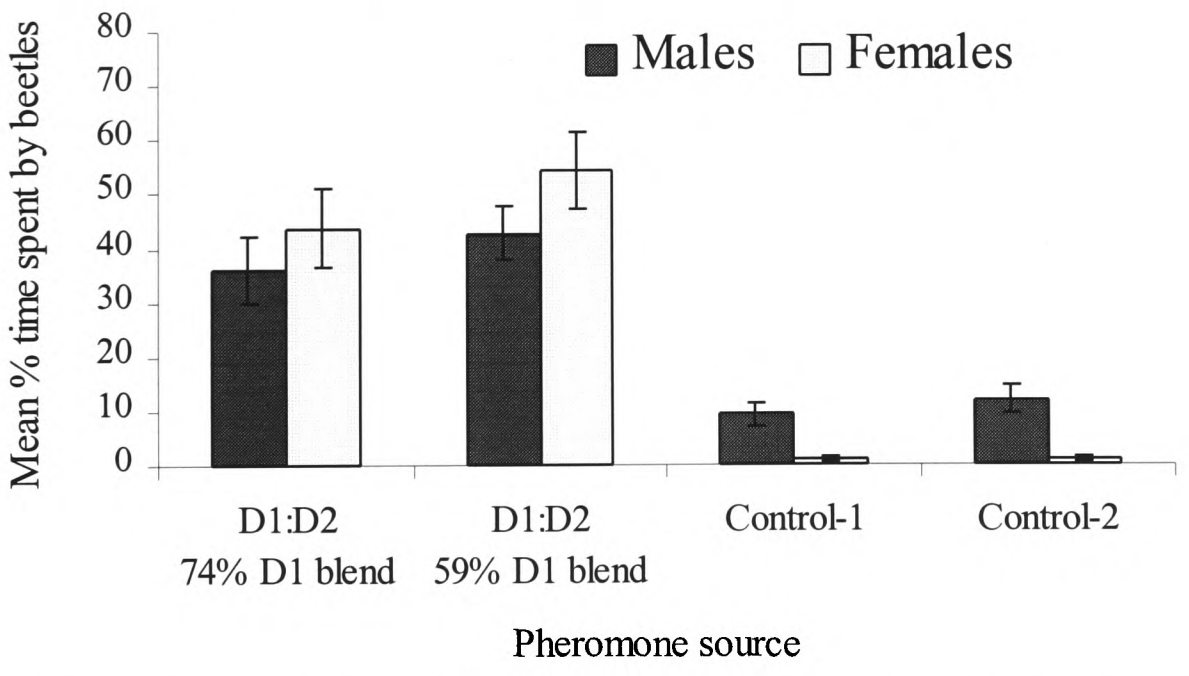


Figure 8.6: Mean percentage of time spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving volatiles from synthetic pheromone blend of different ratios of component or clean air; odour sources were replicated eight times and response of five males and five females was tested for each replication; error bars represent standard error (n=40)

Table-8.4: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from synthetic pheromone sources or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females	
	Z	p-value	Z	p-value
74% D1 blend *vs* 59% D1 blend	-0.46	0.65	-0.20	0.84
74% D1 blend *vs* Control-1	-2.96	<0.01	-4.24	<0.001
74% D1 blend *vs* Control-2	-2.71	<0.01	-4.29	<0.001
59% D1 blend *vs* Control-1	-4.42	<0.001	-4.35	<0.001
59% D1 blend *vs* Control-2	-4.64	<0.001	-4.35	<0.001
Control-1 *vs* Control-2	-0.34	0.74	-0.77	0.44

Is there any difference in response between males and females?

There was no significant difference in response between males and females to 74% D1 blend or 59% D1 blend (Figure 8.6).

The percentage of time spent by males or females in both the odour zones combined, indicated that females (98%) showed significantly higher level of response ($z = -5.93$, $p<0.001$, $n = 40$) than males (79%).

There was no significant difference between males (83%) and females (92%) in the level of discrimination between odour sources.

Greater quantity of pheromone* vs *smaller quantity of pheromone

Mean quantity of pheromone released from synthetic pheromone sources is shown in Figure 8.7 and response of males and females to these releases is presented in

Figure 8.8. There was significant heterogeneity among treatments for males (Chi-square=49.59, df=3, p<0.001) and females (Chi-square=63.32, df=3, p<0.001). However, subsequent pairwise comparisons between treatments demonstrated no significant difference between responses to greater quantity or smaller quantity of pheromone for males or females (Table-8.5).

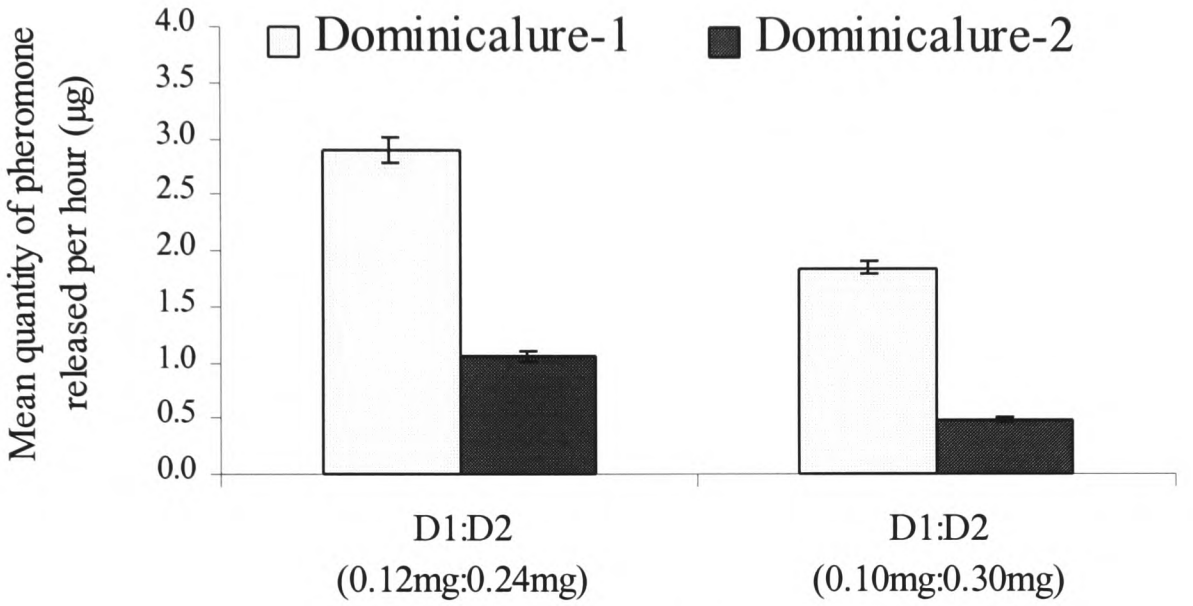


Figure 8.7: Mean quantity of pheromone released from synthetic pheromone sources in one hour; error bars represent standard error (n = 8)

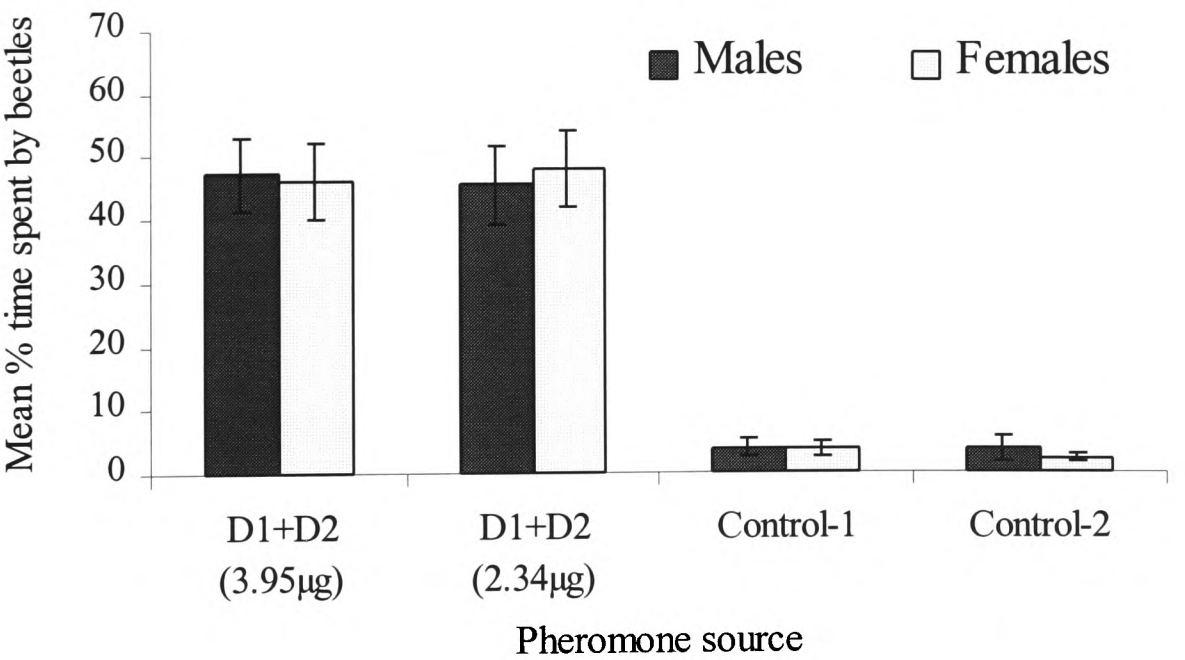


Figure 8.8: Mean percentage of time spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving volatiles from synthetic pheromone blend of different ratios of component or clean air; odour sources were replicated eight times and response of five males and five females was tested for each replication; error bars represent standard error (n=40)

Table-8.5: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from synthetic pheromone sources or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females	
	Z	p-value	Z	p-value
Greater quantity *vs* Smaller quantity	-0.12	0.90	-0.28	0.78
Greater quantity *vs* Control-1	-4.74	<0.001	-4.72	<0.001
Greater quantity *vs* Control-2	-4.62	<0.001	-4.94	<0.001
Smaller quantity *vs* Control-1	-4.46	<0.001	-4.74	<0.001
Smaller quantity *vs* Control-2	-4.17	<0.001	-4.97	<0.001
Control-1 *vs* Control-2	-0.33	0.74	-1.29	0.20

Is there any difference in response between males and females?

There was no significant difference in response between males and females to the greater quantity or smaller quantity of pheromone (Figure 8.8).

The percentage of time spent by males or females in both the odour zones combined, indicated that females showed a slightly higher level of response (94%) than males (91%), however, the difference was not significant.

Males showed slightly higher level of discrimination between odour sources (71%) than females (66%), however, as in the earlier case the difference was not significant.

Pheromone blend* vs *pheromone component D1

Mean quantity of pheromone released from synthetic pheromone sources is shown

in Figure 8.9 and response of males and females to these releases is presented in Figure 8.10. There was significant heterogeneity among treatments for males (Chi-square=449.63, df=3, p<0.001) and females (Chi-square=36.28, df=3, p<0.001). Subsequent pairwise comparisons between treatments demonstrated that the response to the pheromone blend was significantly greater than to D1, both for males and females (Table-8.6).

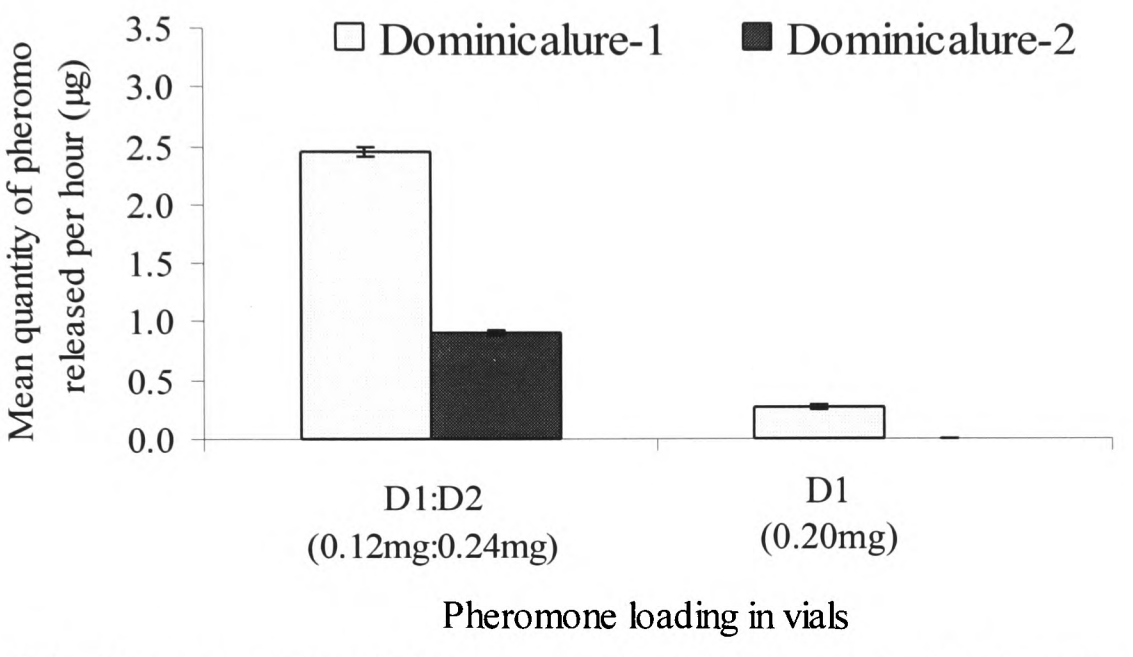


Figure 8.9: Mean quantity of pheromone released from synthetic pheromone sources in one hour; error bars represent standard error (n = 8)

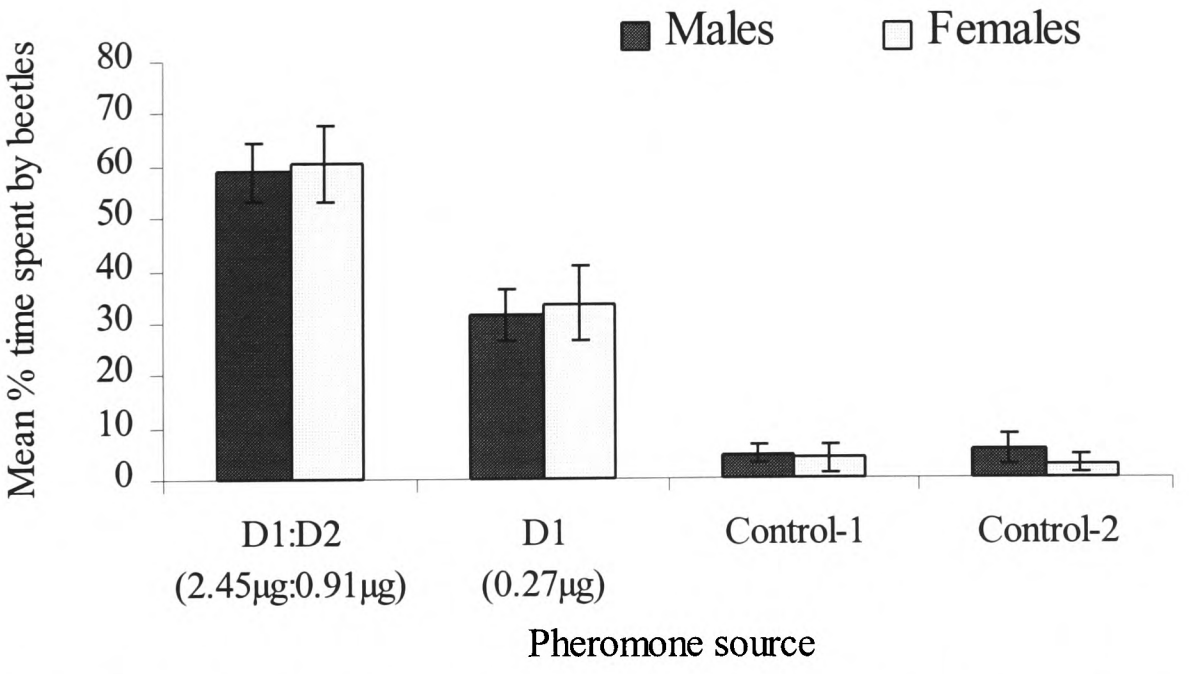


Figure 8.10: Mean percentage of time spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving volatiles from synthetic pheromone sources (D1:D2 or D1) or clean air; odour sources were replicated eight times and response of five males and five females was tested for each replication; error bars represent standard error (n=40)

Table-8.6: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from synthetic pheromone sources or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females	
	Z	p-value	Z	p-value
Pheromone blend *vs* D1	-2.64	<0.01	-2.01	0.045
Pheromone blend *vs* Control-1	-4.97	<0.001	-4.68	<0.001
Pheromone blend *vs* Control-2	-4.44	<0.001	-4.69	<0.001
D1 *vs* Control-1	-3.82	<0.001	-3.31	<0.001
D1 *vs* Control-2	-3.56	<0.001	-3.32	<0.001
Control-1 *vs* Control-2	-0.70	0.49	-0.17	0.87

Is there any difference in response between males and females?

There was no significant difference in response between males and females to pheromone blend or D1 individually (Figure 8.10).

The percentage of time spent by males or females in both the odour zones combined, indicated that females showed significantly ($z = -3.64$, $p<0.001$, $n = 40$) higher level of response (94%) than males (87%).

Females also showed significantly ($z = -3.72$, $p<0.001$, $n = 40$) higher level of discrimination between odour sources (91%) than males (67%).

Pheromone blend* vs *pheromone component D2

Mean quantity of pheromone released from synthetic pheromone sources is shown in Figure 8.11 and the response of males and females to these releases is presented

in Figure 8.12. There was significant heterogeneity among treatments for males (Chi-square=41.96, df=3, p<0.001) and females (Chi-square=51.09, df=3, p<0.001). Subsequent pairwise comparisons between treatments for females demonstrated that response to the pheromone blend was significantly greater than that to D2. However, there was no significant difference for males (Table-8.7).

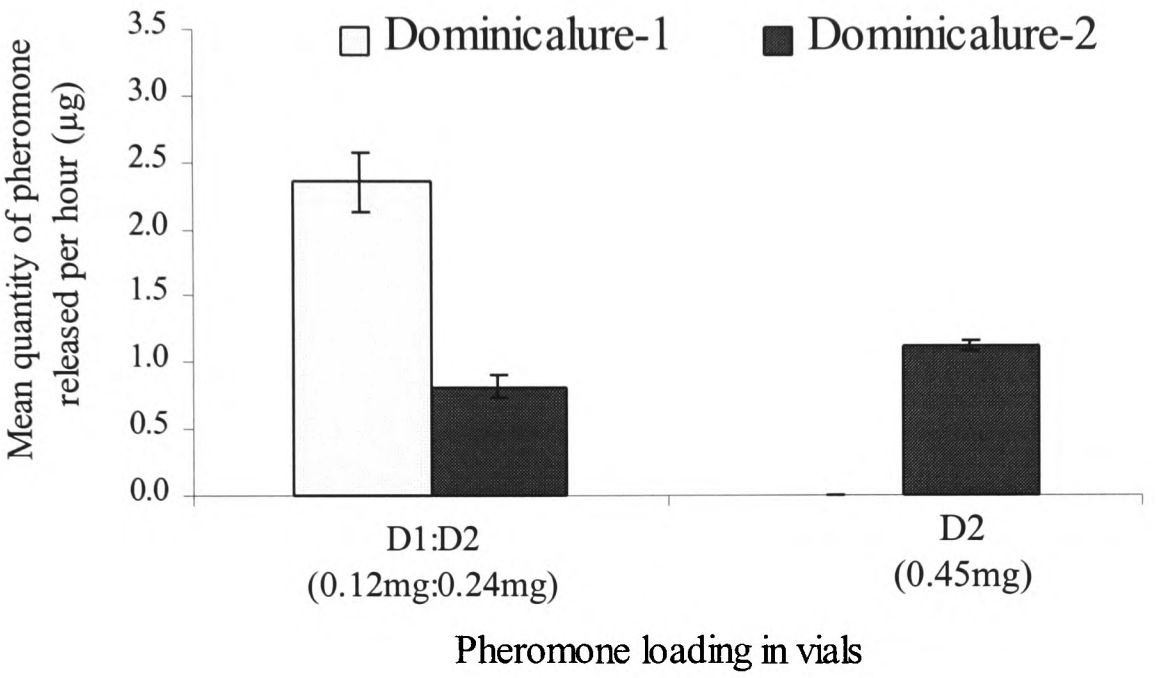


Figure 8.11: Mean quantity of pheromone released from synthetic pheromone sources in one hour; error bars represent standard error (n = 8)

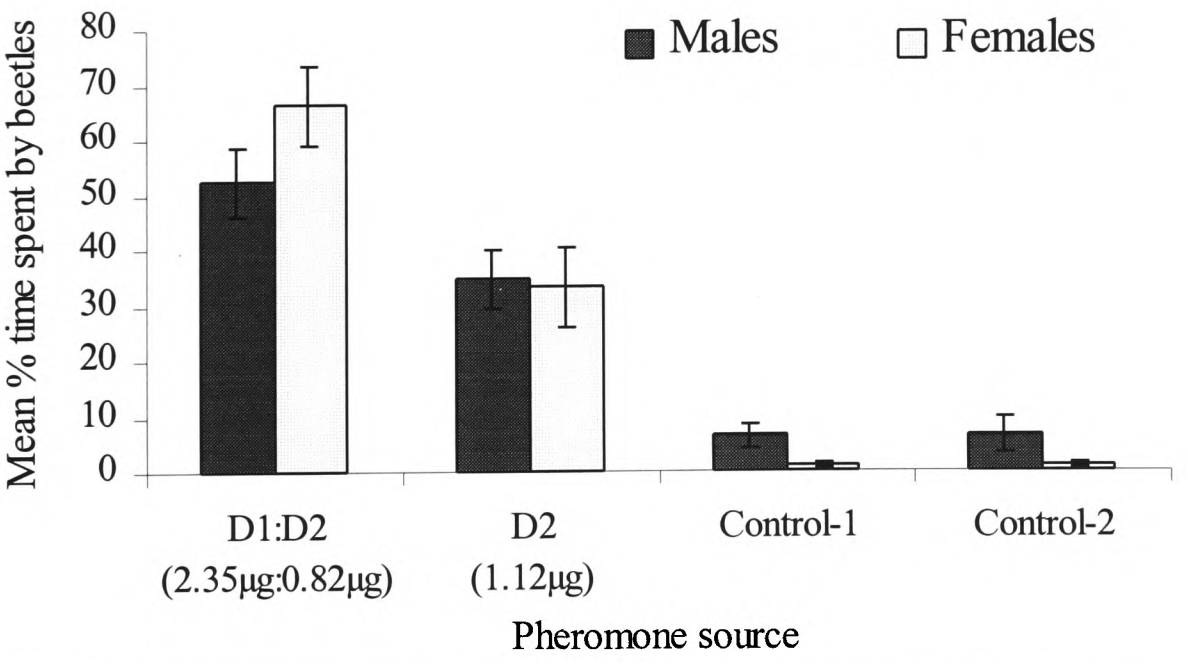


Figure 8.12: Mean percentage of time spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving volatiles from synthetic pheromone sources (D1:D2 or D2) or clean air; odour sources were replicated eight times and response of five males and five females was tested for each replication; error bars represent standard error (n=40)

Table-8.7: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from synthetic pheromone sources or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females	
	Z	p-value	Z	p-value
Pheromone blend *vs* D2	-1.67	0.09	-2.39	0.02
Pheromone blend *vs* Control-1	-4.56	<0.001	-5.01	<0.001
Pheromone blend *vs* Control-2	-3.93	<0.001	-5.04	<0.001
D2 *vs* Control-1	-3.71	<0.001	-3.62	<0.001
D2 *vs* Control-2	-3.48	<0.001	-3.62	<0.001
Control-1 *vs* Control-2	-0.24	0.81	-0.74	0.46

Is there any difference in response between males and females?

There was no significant difference in response between males and females to pheromone blend or D2 individually (Figure 8.12).

The percentage of time spent by males or females in both the odour zones combined, indicated that females showed significantly ($z = -4.80$, $p<0.001$, $n = 40$) higher level of response (99.70%) than males (84.68%).

Females also showed significantly ($z = -3.82$, $p<0.001$, $n = 40$) higher level of discrimination between odour sources (71%) than males (93%).

Pheromone component D1* vs *pheromone component D2

Mean quantity of pheromone released from synthetic pheromone sources is shown in Figure 8.13 and response of males and females to these releases is presented in

Figure 8.14. There was significant heterogeneity among treatments for males (Chi-square=43.10, df=3, p<0.001) and females (Chi-square=49.82, df=3, p<0.001). Subsequent pairwise comparisons between treatments demonstrated that response to pheromone component D2 was significantly greater than that to D1, both for males and females (Table-8.8).

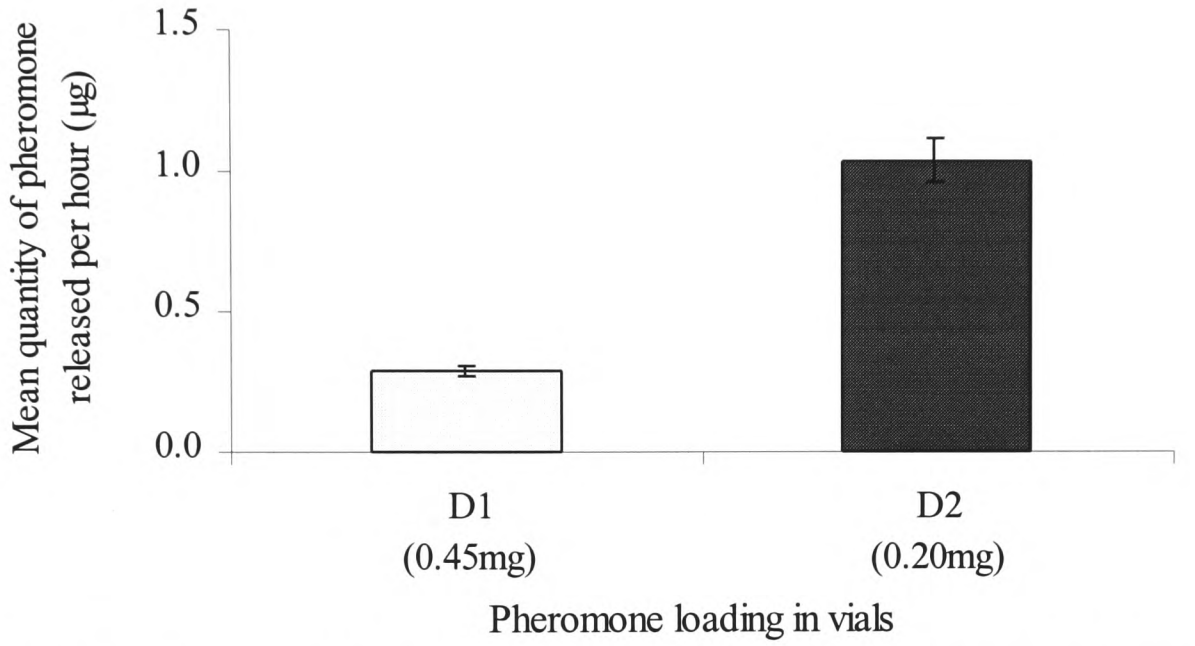


Figure 8.13: Mean quantity of pheromone released from synthetic pheromone sources in one hour; error bars represent standard error (n = 8)

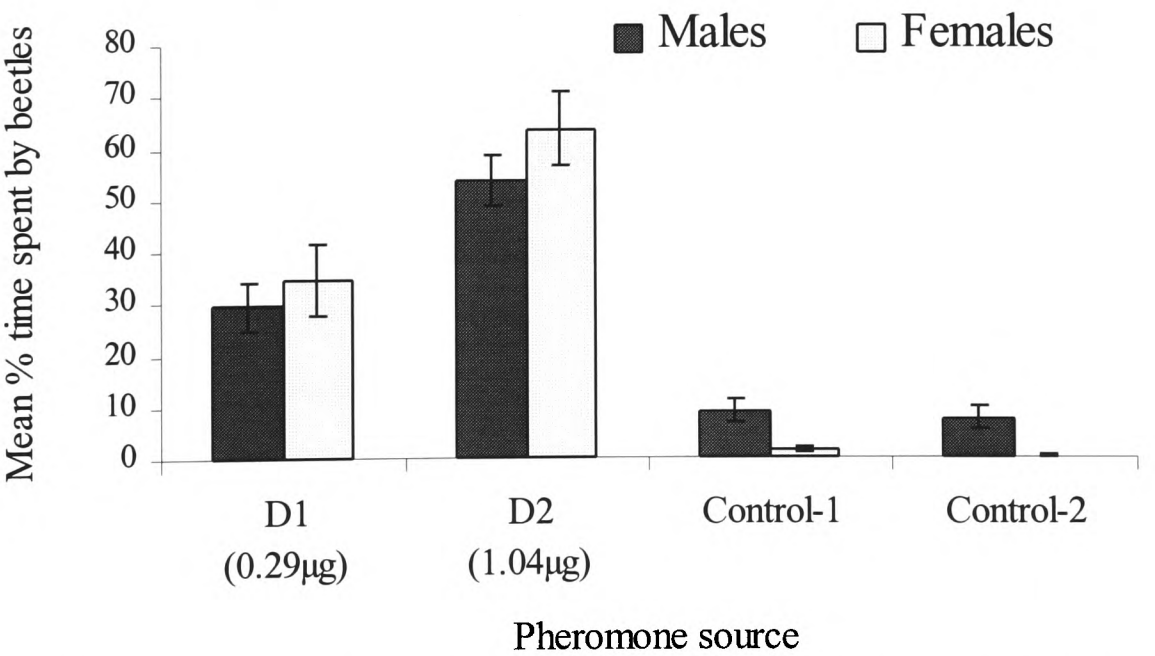


Figure 8.12: Mean percentage of time spent by adult *R. dominica* in different odour zones of a four-choice airflow olfactometer receiving volatiles from synthetic pheromone sources (D1 or D2) or clean air; odour sources were replicated eight times and response of five males and five females was tested for each replication; error bars represent standard error (n=40)

Table-8.8: Pairwise comparisons between the percent time spent by beetles in different zones of a four-choice airflow olfactometer receiving volatiles from synthetic pheromone sources or clean air (control); data were analysed using Wilcoxon test for two-related samples

Treatment comparisons	Males		Females	
	Z	p-value	Z	p-value
D1 *vs* D2	-2.17	0.03	-2.15	0.03
D1 *vs* Control-1	-3.26	<0.001	-3.80	<0.001
D1 *vs* Control-2	-3.85	<0.001	-3.80	<0.001
D2 *vs* Control-1	-5.28	<0.001	-4.96	<0.001
D2 *vs* Control-2	-4.89	<0.001	-4.96	<0.001
Control-1 *vs* Control-2	-0.28	0.78	-2.22	0.03

Is there any difference in response between males and females?

There was no significant difference in response between males and females to D1 or D2 (Figure 8.14).

The percentage of time spent by males or females in both the odour zones combined, indicated that females showed significantly ($z = -5.55$, $p<0.001$, $n = 40$) higher level of response (98%) than males (83%).

Females also showed significantly ($z = -3.32$, $p<0.001$, $n = 40$) higher level of discrimination between odour sources (88%) than males (63%).

8.4. DISCUSSION

8.4.1. Pheromone production by *R. dominica* males at different times of day

Pheromone entrainments have shown that mean quantities of pheromone produced by *R. dominica* males in different two hour-periods from 08.00h to 16.00h were very similar early on in the series but there was a considerable increase in the quantities of pheromone produced during the dusk/evening hours from 16.00h to 20.00h. One-way ANOVA did not show any significant heterogeneity between the quantities of pheromone produced during various two-hour periods. A pairwise comparison of the treatments was made because mean quantities of pheromone components produced by group of twelve males in the last two-hour light period (18.00h-20.00h) (D1=1.03µg, D2=0.98µg) were nearly double that produced in first two-hour period (0.800h-10.00h) (D1=0.60µg, D2=0.60µg). This was a considerable difference, and pairwise analysis also confirmed that the quantities of pheromone produced in the evening hours (16.00h-20.00h) were significantly greater than those produced during morning hours (0.800h to 16.00h).

Pheromone quantities produced per hour in the first eight hours of photophase period (0.800h to 16.00h) were fairly constant. Vick *et al.* (1973) reported similar results for females of *Trogoderma inclusum* LeConte and *T. glabrum* Herbst where pheromone production during first nine hours of photophase was similar but they did not entrain for the reminder of the day. Peak period of pheromone production by *R. dominica* males in the present studies was towards the evening hours that coincides with its peak period of flight activity which has been reported as before dark/dusk (Barrer *et al.*, 1993; Wright and Morton, 1995). The ratio of two

pheromone components D1 to D2 was nearly the same at different times of day. Opposite results were found for oblique-banded leafroller, *Choristoneura rosaceana* (Harris) in which ratio of pheromone components was effected by the time of day (Delisle and Royer, 1994).

The mean quantity of the pheromone produced per male per hour during night time (20.00h to 08.00h) was similar to that produced during twenty four-hour period. It is therefore, quite possible that production of the pheromone has the same pattern as during day time. It may be assumed that pheromone production is at its peak hours around dusk and at other times of day its is fairly constant (Fig 8.15), however further studies are needed to confirm this.

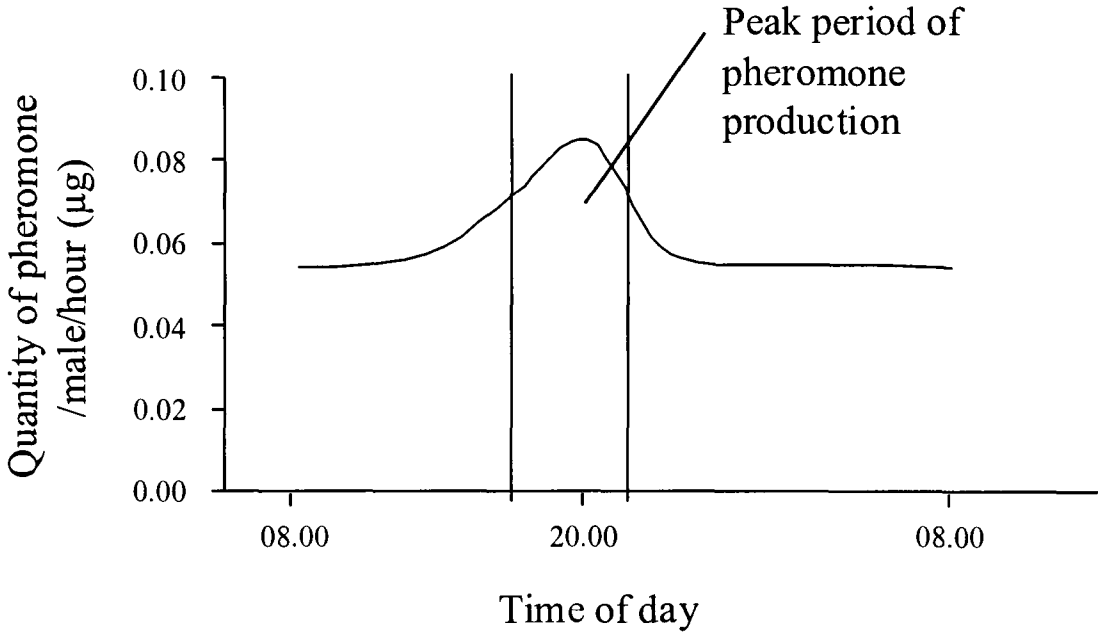


Figure 8.15: Hypothetical curve showing pheromone production by *R. dominica* males at different times of day under laboratory conditions of 27±1 °C, 64±5% r.h. and twelve hour light/dark cycle (08.00h-20.00h light, 20.00-0.800 darkness)

The quantities of pheromone released by *R. dominica* males were similar from 08.00h to 16.00h, it was therefore, decided to perform the experiments within these time limits.

8.4.2. Preference of *R. dominica* adults for natural or synthetic pheromone sources

The quantity of pheromone component D2 released from synthetic pheromone source (0.19µg/hour) was similar to that released by *R. dominica* males (0.18µg/hour). However, the quantity of D1 was greater for synthetic source (0.31µg/hour) than for the males (0.24µg/hour). The results of the olfactometer tests showed that synthetic pheromone was equally attractive as the pheromone released by males. Male beetles showed similar responses to both the pheromone sources while females showed a slightly higher response to the pheromone released by males (Figure 8.4). Burns and Teal (1989) reported that virgin females of noctuid, *Hydraecia micacea* (Esper) were more attractive to the males than synthetic pheromone.

The response to the naturally-produced and synthetic pheromone was similar albeit that the synthetic pheromone had a slightly greater amount of D1 in the blend. This suggests that there is little or no difference in the attractive properties of these two sources and confirms that synthetic pheromone can be used reliably in experiments to explore response of beetles to modified pheromone signals.

8.4.3. Which characteristics of a pheromone signal affect the beetle's response?

The pheromone blends released from polythene vials loaded with different weights of synthetic pheromone had the release ratios and quantities different from those that had been predicted. Therefore, the main objective of this study, to determine

which characteristics of the pheromone signal of *R. dominica* males are associated with the observed responses from conspecifics was not accomplished. However, the results do provide some information about the beetle's behaviour in response to pheromone signals. When the 74% D1 blend was tested against the 59% D1 blend, the beetles showed slightly higher attraction for 59% D1 blend despite the fact that this had a smaller release rate of pheromone (2.05μg/hour) than the 74% D1 blend (2.53μg/ hour). When the greater release rate of pheromone (74% D1 in the blend) was tested against the lower release rate of pheromone (79% D1 in the blend), the beetles showed similar attraction for both the pheromone signals. This suggests, that above a certain level the quantity of the pheromone does not have much additional effect on beetle's response. Likewise, the ratio of the pheromone components also does not seem to have much effect within a certain range. The range tested here was from 59% of D1 to 79 % of D1, which is not much different from the blends produced by the males throughout these studies under favourable conditions (see Chapter 5 to 7). Therefore, it is logical to suggest that the beetle response is affected only when the difference in ratios is outside the 'normal' range.

The beetles were significantly more attracted to pheromone blends than to individual components but as the quantities of individual components were much smaller than those of blends, this may not be a valid comparison. It has been reported for some insects that mixtures of pheromone components are more attractive than individual components (Quartey and Coaker, 1993; Valeur and Lofstedt, 1996). In the current tests, a much lower quantity of D1 (0.27μg/hour) (Figure 8.12) produced a similar response to a higher quantity of D2 (1.12μg/hour) (Figure 8.10) when these individual components were compared with the mixture.

In this case the composition of the mixture was almost identical in the two tests. When D1 and D2 were tested directly against each other, D2 was significantly more attractive than D1. However, in this case the quantity of D1 released was only 28% of D2 yet D1 managed to attract 54% as many beetles. This suggests that an equal release rate of D1 may be more attractive than D2, at least in the confines of an olfactometer. Cogburn *et al.* (1984) reported that traps baited with different ratios and individual components of pheromone of *R. dominica* were equally attractive. However, these results are not comparable with present studies because Cogburn *et al.* (1984) did not make direct comparisons between different pheromone lures. It has been reported for the related species *Prostephanus truncatus* that pheromone component Trunc-call 2 individually or in combination with the other component Trunc-call 1 is significantly more attractive than Trunc-call 1 alone for the flying beetles. While the mixture of the two is significantly more attractive than either of the components individually for the walking beetles (Hodges *et al.*, 1998).

Difference in the response between males and females

In all the tests, females showed significantly higher levels of response to pheromone (responses to both pheromone sources combined) than males but there were no significant differences between males and females in response to the same treatment. Females also showed significantly higher level of discrimination between pheromone sources, when blends were compared with the individual components or individual components with each other. These differences were not significant when different pheromone blends were tested against each other.

Similar differences between males and females were also found earlier in these studies (see Chapter 4 and 6) when their response to natural pheromone (males) was tested. This suggests that the primary function of the aggregation pheromone of *R. dominica* is as a female-attractant. This will be discussed in more detail in Chapter 9.

Chapter:9

General Discussion

9.1. Review

The overall aim of this study was to gain insights into the host-finding behaviour and pheromone communication system of *R. dominica* and the interactions between the two. There are remarkably few studies of host finding by *R. dominica* (Crombie, 1941; Dowdy *et al.*, 1993). Although the male-produced pheromone has been characterised and studied as lures in traps (Sinclair *et al.*, 1984; Rejesus and Butuanon, 1988; Trematerra and Daolio, 1990; Fargo *et al.*, 1994), there have been only superficial studies of factors affecting its production (Mayhew and Phillips, 1994). The aggregation pheromone is instrumental in food source and mate location, but for *R. dominica* the relative importance of these roles for signaller and responder and the interactions of pheromone production and host selection have not been explored. The present work provides a detailed study of these aspects of host finding and pheromone production and takes account of the inevitable interactions between the two aspects of behaviour. Furthermore, previous studies with *R. dominica* and indeed other insects have tended to focus either on chemical measurement of the quantity and quality of the pheromone production or on the behavioural response of beetles to natural and/or synthetic pheromone. The present study utilises both approaches and is able to correlate the two and provide much greater insights into the biological consequences of treatment effects of pheromone production.

The response of adult beetles to different host-grain volatiles alone and in combination with aggregation pheromone was studied. Different aspects of pheromone signalling by males such as individual variation, association of the rate of pheromone release with rate of feeding/boring and pheromone production at different times of day, were investigated. Different factors that might cause a modification in pheromone signalling by *R. dominica* males such as host-type and presence of conspecifics and the responses of conspecifics to these modified signals were studied. An attempt was made to determine which characteristics of a pheromone signal are most correlated with the observed responses. The results have been discussed in detail at the end of relevant chapters. Here, these results are drawn together to give an overall picture of the pheromone communication system and host finding behaviour of *R. dominica*, and also to discuss these findings in the broader perspectives of the evolution of pheromone communication and potential practical applications.

9.2. Host selection by *R. dominica* – determination of host suitability

The present study aimed to investigate the role of host volatiles in the primary selection of a food source in *R. dominica*. Behavioural responses of adult beetles towards different host-grain volatiles were investigated to determine whether this insect could distinguish between the volatiles of one host (suitable) from others (less/unsuitable). The findings suggested that although *R. dominica* adults respond to host volatiles, they are not able to select a suitable host on the basis of host volatiles alone, as they were more strongly attracted to groundnut (unsuitable/non-host) volatiles than to wheat or maize (suitable hosts) volatiles. The beetles also did not show any preference for the odour of their development medium over the

odour from a potential host they had not yet experienced. This raises the question, if the beetles are not able to determine the suitability/quality of a host from its volatiles then are there any other host-based semiochemical cues that may be used by beetles for this purpose?

Rhyzopertha dominica males produce pheromone only when present on a food source (Mayhew and Philips, 1994), therefore, the production of pheromone signals in this insect indicates to conspecifics the presence of an available food source. However, it was not known whether the pheromone signals contain any information about the quality or suitability of the food source. To investigate this, responses of the beetles towards host-grains (wheat, maize and groundnut) were observed when male *R. dominica* were also present on them. It was hypothesised that if the pheromone signals released from males on host-grains do not contain any information about the quality/suitability of the food, then a combination of groundnut volatiles and aggregation pheromone (beetle-infested groundnut) would get a stronger positive response than a combination of wheat or maize volatiles and aggregation pheromone. This is because groundnut volatiles on their own were more attractive than wheat or maize volatiles. In fact, beetles preferred a combination of host (wheat or maize) volatiles and aggregation pheromone over the non-host groundnut volatiles/pheromone mixture. This suggested that, either pheromone signals are not produced by the beetles on non-host groundnut or the signals are less attractive to responders than those produced on hosts wheat or maize. Further experimentation demonstrated that *R. dominica* males would produce pheromone signals even when present on groundnut or de-oiled groundnut. However, the pheromone signals produced by males on this unsuitable host differed from those produced on a suitable host (wheat or maize). In one

experiment, the quantity of pheromone component Dominicalure-2 (D2) was not affected by the host-grain type, but the quantity of Dominicalure-1 (D1) produced by males present in groundnut (normal or deoiled) was significantly reduced. In the second experiment, quantities of both the components were affected, but as in the earlier case the greater effect was on the quantity of D1. This altered the ratio of the two components significantly reducing the proportion of D1 in the pheromone blend. As the rate of pheromone release from males was found to be positively associated with the rate of feeding/boring (measured by the quantity of dust produced by individual males), it might be argued that modified pheromone signals are not a response to the nutritional suitability of a host but are simply due to different feeding/boring rates in different hosts. This would result in differences in the quantity of pheromone released. However, it is worth noting that different feeding/boring rates were correlated with the absolute amounts of pheromone components but not with change in the ratio of the two. Since change in host type resulted in a very different pheromone ratio, it is suggested that the host type influence on signalling in not simply due to differences in feeding/boring rate.

It is worth comparing the host selection methods of *R. dominica* and *P. truncatus*. These two are taxonomically closely related, and have very similar boring habits. *P. truncatus*, is believed to live largely as a wood borer attacking wood of appropriate moisture and starch content (Nang'ayo, 1996). It has been found in the distal portions of branches and twigs girdled by cerambycids (Borgemeister *et al.*, 1998) and can be trapped in very large numbers many kilometers away from agricultural land or grain stores (Nang'ayo *et al.*, 1993). Host volatiles appear to play little or no role in helping this species find its stored-products hosts, maize and dried cassava roots (Hodges, 1994; Scholz *et al*, 1997b; Fadamiro *et al.*, 1998).

There is strong evidence to suggest that *P. truncatus* is still a relatively poorly adapted storage pest as primary host-selection occurs by chance (Hodges *et al.*, in press). In contrast, *R. dominica* showed a strong reaction to volatiles from hosts and even non-host plants in this study which supports field observations by Barrer (1983) suggesting that the location and selection of grain by this species is not a matter of chance. *Rhyzopertha dominica* appears to be better adapted as a pest of grain than *P. truncatus* although its abilities to actually discriminate hosts and non-hosts is worthy of further study in view of its strong attraction to groundnuts on which it could not breed.

9.3. Biological role of aggregation pheromones

These studies have shown that *R. dominica* males produced smaller quantities of pheromone when present with females than when they were alone. Both the pheromone components D1 and D2 were affected although D1 was more affected causing a modification in the ratio of D1 to D2. Mayhew and Phillips (1994) did not observe any significant difference in the level of the pheromone release between males of *R. dominica* present alone or paired with females. The possible reasons for the difference in the results of Mayhew and Phillips (1994) and the present studies has been discussed in detail in Chapter 7.

Study of the pheromone output by individual males showed that in most cases, pheromone signals were modified in the presence of females. The presence of females resulted in a lowered emission of the pheromone components particularly of D1. There was however, no consistency between males in the magnitude of the change in the pheromone output when males were confined and separated from direct contact with the females.

This raises the questions, why does the male modify its signal in the presence of females? Does this give any message to the receiving beetles? Is this message for receiving conspecific males or females or both? The results of olfactometer tests showed that the beetles clearly preferred the pheromone signals released by lone males to those released by the males with females. In bark beetles, the reduced production of pheromone and release of pheromone by males after mating appears to play a major function in the process of terminating the aggregation phase of host colonisation (Byers, 1981). On the basis of the current results, it can be hypothesised that a male signaller in the presence of females produces a modified pheromone signal to make itself or its food source less attractive for the responding beetles and hence attempts to limit or reduce the rate of arrival of new individuals. *Rhyzopertha dominica* is a polygamous insect, therefore, making itself considerably less attractive after mating with or in the presence of one female might be a disadvantage. It should try to mate with as many females as possible in order (according to the theory of natural selection at the level of the individual Davies and Krebs, 1978; Alcock, 1982), to maximise the number of individuals in the next generation carrying its genes. But in that attempt it should not over populate its habitat in a way that may reduce the survival prospects of its offspring due to the over utilisation of the food source (Peter and Barbosa, 1977). Therefore, there may be a considerable advantage in ceasing pheromone production or modifying the pheromone signal (to limit the arrival of new individuals) when an optimum number of females has been attracted. This explanation of the modified pheromone signals by *R. dominica* suggests that the pheromone signals produced by the males are meant to be for the females. If that is the case then why are males attracted to these signals?

Male signallers may attract females by announcing the presence of a suitable mate or by advertising the availability of a suitable food source, as *R. dominica* produces its pheromone only in the presence of food. Females who have the ability to choose between two different males on the basis of their pheromone signals (Birkinshaw, 1998) may discriminate between male signallers to find a mate and/or to locate a suitable food and oviposition site (Svensson, 1996). Hodges and Dobson (1998) reported that unmated females of the related species *Prostephanus truncatus* will not leave food to locate a signalling male, emphasising in this case the prime importance of the signal as a means of host selection. It is possible that female choice for a signalling male *R. dominica* is based on host selection since the production of pheromone announces the presence of food. Other males may exploit these signals for their own benefits i.e. (1) to find a suitable food source (Schlyter and Birgersson, 1989) and (2) to enjoy increased chances of finding suitable mates (Burkholder and Ma, 1985).

Boughton and Fadamiro (1996) argued that if the main function of male-produced pheromones is to attract females, then females should show a significantly higher level of response to the pheromone signals than males. In present studies, in a few experiments female response to pheromone signals was significantly greater than males while in almost all the other experiments it was greater although not significant. Scholz *et al.* (1998) reported that in a four-choice olfactometer, females of *Prostephanus truncatus* were significantly more attracted to synthetic pheromone than males. In the same species a greater but not significantly different response from females was also observed in field trapping experiments (Scholz *et al.,* 1997a; Hodges *et al.,* 1998). The reason why there is not a huge difference between the responses of males and females may be that, during the course of

evolution, males have developed an efficient mechanism to receive and interpret the pheromone signals (which evolved to attract females for mating) to enhance their fitness.

A communication system will be maintained (and further elaborated) if both the signaller and the responder gain from the interaction (Alcock, 1982). If it is the case that male-produced (aggregation) pheromone signals are meant to be for both the sexes, then responding males will benefit by finding a suitable food source but what benefit will signalling males get by attracting other males? Obviously the arrival of the new males will increase competition for mates and food. In contrast, the arrival of females benefits both the signalling male (by finding mates) and the responding females (by finding suitable food source and/or mates). One possible reason often given is that the arrival of other males may help the signalling male to overcome the host resistance (Borden, 1982). This may be the case for some of the bark beetles that attack living trees. They need to quickly overcome the resistance mechanism of the tree and to kill it so it cannot produce any more toxic materials in its defence (Byers, 1995). In storage conditions where the host-plant is dormant and is not known to produce any toxin in its defence, calling for other individuals to help in attack does not seem likely. In *R. dominica,* when a male starts producing pheromone he is already feeding (boring), this means he has already overcome the physical resistance of the host and if there were any toxins he has already become the target of those. Now how will the arrival of other individuals (males) benefit the signaller? Even if the signaller does require some help, females could fulfil this purpose and he does not need to call for potential competitors (other males).

9.4. Energy conservation or alternative male strategy?

Males present in groups produced smaller quantities of pheromone (per male) than single males. Similar results were reported by Mayhew and Phillips (1994) and Dowdy *et al.* (1993). Unlike the presence of females, which showed a marked effect on the ratio of the pheromone components produced by *R. dominica* males, the presence of conspecific males apparently did not show any effect on the ratio of the pheromone blend. As pheromone production can not be quantified from individual males when present in groups, one cannot be sure whether every individual in a group is producing less, or few are producing a normal quantity and few are not producing at all. If every individual in the group is producing less, it could be a strategy by individual males to conserve the energy required for pheromone biosynthesis (Mayhew and Philips 1994), as pheromone synthesis is likely to be an energy consuming process (Schlyter and Birgersson, 1989). If some males are not releasing pheromone at all then it is possible that they are simply exploiting the signals of the other males. Cade (1980) has reported this alternative strategy in males of field cricket (*Gryllus*) that do not sing to attract females and exploit the sexual signals (chirping) of other calling males to get mates. Another possibility for why some males may not be signalling has been demonstrated by Moore *et al.* (1995) in cockroaches. In this case, only dominant males produce pheromone (Moore, 1998). The subordinate (less attractive) males will not produce pheromone because they will not have any chance to attract a mate in the presence of a more attractive male. The dominant male cannot maintain the same (higher) quality or quantity of pheromone release for more than a few weeks, probably because this taxes his resources. The subordinate males then have the

chance to take over and become dominant but they can only do so if they don't waste their efforts at the time when they can not compete successfully.

9.5. Potential practical importance and application of these findings

1. The processes of selecting a suitable host and pheromone communication are very important components of an insect's biology. The information that has been provided in this thesis about different aspects of the function of these systems together with the existing knowledge could be used to interrupt these natural processes, this would provide a sound basis to develop environmentally sustainable control methods.

2. Pheromone signals produced by males on an unsuitable host (groundnut) were different than those produced on a suitable host (maize). Responding beetles showed greater attraction for signals produced on the suitable host (maize). This information presents us with clues about the composition of the pheromone that would be most effective as a lure in traps

3. Non-host groundnut volatiles were more attractive to beetles than hosts wheat or maize. The beetles showed greater attraction for mixtures of host volatiles and aggregation pheromone. This indicates that a trap with a combination of groundnut volatiles and pheromone may be more efficient than a combination of wheat or maize and pheromone.

4. The differences in the response of beetles to different odour sources when presented singly or at the same time suggests that beetle's choice for an odour source might depend on the choice available. It is therefore, important that pest

managers consider the odour context in which they might attempt to manipulate beetle behaviour using semiochemicals.

5. The results have indicated that the response of beetles to pheromone does not increase with the quantity of pheromone after a certain threshold level. This means that using greater quantity/dose of pheromone in traps may not necessarily increase its efficiency.

6. In the present studies, attraction of the beetles to single male signallers demonstrates the ability of a single beetle to start infestation in a store.

7. The information provided about the pheromone release rate at different times of day would help to plan experiments where *R. dominica* males are required to be used as source of pheromone.

9.6. Suggestions for future work

The findings of the present study suggest the following avenues for future research:

1. Investigation of behavioural response of *R. dominica* to a wide range of host/non-host plant volatiles should be undertaken to establish whether the beetle is generally unable to discriminate between suitable and unsuitable hosts. This will also help to determine which host volatiles would be best to combine with synthetic pheromone to maximise trap catch.

2. Further investigation of combinations of host volatiles and synthetic pheromone to give a clearer picture of how these work together to increase beetle response.

3. Further comparisons of the pheromone blend composition released by *R. dominica* on different host/non-host plants to confirm the current findings and document the extent to which signals may be modified. A range of groundnut varieties, from those unsuitable as a host to those that will support significant population development, would offer a good basis for such a study if such varieties could be found. Alternatively, an artificial host could be developed that could be prepared at a range of different nutritional values.

4. Studies to establish which characteristics of the pheromone blend make it more or less attractive. This will involve further work with synthetic pheromone.

5. Investigations of the biosynthetic pathway of the two components to establish the mechanism by which the composition of the pheromone blend released by males is affected by external factors such as host-type or presence of females. Such studies might also give clues as to the energetic and nutritional constraints on signalling that would help to interpret evolution of the pheromone signals more clearly.

6. More detailed investigations of the individual and combined roles of D1 and D2 in beetle response, including electro-antennography to complement bioassay work. This will hopefully shed some light on the significance of reducing the proportion of D1 in the blend and together with 3., 4. and 5. above, may allow further speculation of the function and evolution of the aggregation pheromone of this species.

7. Further studies on pheromone output of males in groups to determine the factors that result in them producing less pheromone than single males.

8. A search for the site of production and release of pheromone within the beetle.

9. Key aspects of the current study should be repeated using flying rather than walking beetles. It is possible that there are significant differences in the response of flying beetles to modified pheromone signals and pheromone and host odour mixtures. The need for speed of reaction are probably more critical at this stage of the beetle's distribution behaviour. It is likely that only when flying beetle response has been investigated will the full significance of the modified pheromone blend be revealed.

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