STUDIES ON THE BEHAVIOUR OF MALES OF CALIFORNIA RED SCALE AONIDIELLA AURANTII (MASKELL)

by

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SUMMARY

California red scale, Aonidiella aurantii (Maskell), is the most important pest of citrus in Australia, California and the Mediterranean countries.

Both the male and female are active following emergence during the wingless "crawler" stage of the first instar. After wandering for a few hours on the host plant the crawler begins to feed and to form a waxy scale covering. The female does not move again; the male is again active for a short time as an alate adult.

Observation indicated that almost all the males of red scale emerge during the afternoon and are dead by the next morning. Mortality commenced 3 h after emergence, about half the males were dead within 5-6 h, and all were dead within 12 h. Copulatory activity occurs shortly after emergence.

Environmental factors such as temperature, relative humidity and light may affect male emergence and longevity. With an increase in temperature the distribution of emergence was shifted closer to midday i.e. the peak emergence at higher temperature occurred earlier than at lower. The time of peak emergence was earlier at higher light intensity and was delayed by lower light intensity. The daily rhythm of emergence of male is entrained by an interaction between the light and temperature cycles. The light is apparently the critical cue for the release of emergence, with darkness or extreme high and low temperature inhibiting it. Emergence was delayed at higher humidity, a result of the accumulation of moisture in the waxy scale which prevented free emergence. The longevity of male was longest at the lowest light intensity and temperature.

Regulation of mating activity of red scale is affected by both light

and temperature. Copulatory activity of males was found to increase with light intensity and temperature.

Virgin females emit a highly attractive sex pheromone. Females were attractive from the time the gray margin began to developed (26 days from crawlers at 25°C). They were most attractive during the first week of life.

A special trap was designed to test the responses of flying males to sex pheromone released by virgin females. Responding males were found to be stimulated by the pheromone and to orient upwind and fly towards the source. The effect of wind speeds of 0, 0.5 and 1 m/sec on male behaviour was determined. Observation showed that in nominal wind speeds of 0.5 and 1 m/sec most males flew upwind, whereas at a nominal zero wind speed, there was no significant preference for either direction.

By allowing males on emergence to walk over fluorescent dust as a marker, free flight and pheromone-searching behaviour of the males were observed under ultraviolet light.

The number of males captured by traps baited with living caged females was influenced by the elevation of traps above the soil surface. A greater number of flying male scales were trapped in the middle third of the trees. Wind direction and velocity are also important factors influencing trap capture in the field. Traps placed upwind attracted more males than the traps placed downwind, a larger number of males were attracted to the traps placed 4 and 8 m from their release point than at any other distance in the field.

It is possible that the control of red scale, could be based on the release of sufficient synthetic female sex pheromone spread over large areas to disrupt of male attraction to female.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, contains no material previously published or written by another person, except when due reference is made in the test of the thesis.

(Jwo-Yee Yan)

August, 1985.

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CHAPTER 1

GENERAL INTRODUCTION

The California red scale, Aonidiella aurantii (Maskell), is probably the most important insect pest of citrus in the world (Ebeling, 1959) and, until recently, was considered the most serious citrus pest in Australia, California and the Mediterranean countries.

The control of red scale has so far been achieved mainly with contact insecticides and fumigants, but proven instances of the development of resistance to chemicals used in control systems are increasing. Red scale in California developed resistance to fumigation with HCN in about 1913 (Quayle, 1922; Brown, 1961; Melander, 1914).

The use of insecticides may have other serious drawbacks, such as environmental pollution, public health hazards, reduction of beneficial and non-target organisms. In addition, the rapidly increasing costs of chemical control programmes, in part due to rapid price rises and apparent shortages of petroleum products in recent times, is becoming another limitation against such controls.

Earlier work in California, Australia and South Africa has shown that some measure of control can be achieved by parasites such as Comperiella bifasciata Howard and Aphytis spp. (Flanders, 1943; Flanders, 1951; Debach and Landi, 1959; Debach and White, 1960; Compere, 1961; Bedford, 1968; McLaren, 1971). However, the natural enemies of red scale were found to be more susceptible to insecticides than the red scale. Thus the integration of insecticides and biological control of red scale does not seem possible.

For sereval insect pests, sex attractant traps baited with either living virgin females, sex pheromone extracted from living females, or synthesized sex pheromone, have been used in many areas for insect survey and detection (Dean and Roelofs, 1970; Riedl et al., 1976; Sanders, 1978; Carde', 1980. and Schwalbe, 1981). They are also considered to be of potential use in control by "disruption of communication", and an understanding of how the male finds the female is of great practical importance in the development of these techniques.

Lepidopterous sex pheromones have received a great deal of attention during recent years. This is due in part to economic reasons; the larvae of certain species of Lepidoptera represent some of our most serious agricultural pests. Difficulties that arise in controlling these pests with conventional techniques, mainly insecticidal, have caused an increased demand for more research into alternate means of control, including behavioural manipulation by sex pheromones (Shorey and Gaston, 1967). Also, with the development of modern techniques and instrumentation, the sex pheromones of Lepidoptera have offered exciting substrates to chemists interested in working with submilligram quantities of chemicals and to electrophysiologists interested in exploring mechanisms of olfaction. A number of reviews have appeared in recent years summarizing and analyzing the literature in this field (Kennedy, 1978; Sanders, 1979; Baker and Carde', 1979; Kydonieus and Beroza, 1982).

The problem of insect orientation towards distant odour sources is also intrinsically interesting and much work has been done with wind tunnels to study the flight of moths in attractant plumes (Kennedy and Marsh, 1974; Miller and Roelofs, 1978; Marsh et al., 1978; Carde' and

Hagaman, 1979). These studies have shown that moths fly upwind in a series of largely horizontal zigzags which generally decrease in amplitude as the source is approached.

Several investigators have suggested recently that, if sufficient synthetic females sex pheromones were spread over large areas, causing the air to be permeated to a sufficiently high level, the additional increment of pheromone contributed by wild females of the given species would be imperceptible to males. Thus the males would never find and inseminate the females (Roelofs et al., 1979; Doane and Brooks, 1980; Cardé, 1981; Rothschild, 1982; Sanders and Seabrook, 1982). The proposed method has been termed the mating disruption.

The presence of certain compounds (generally analogues of the natural pheromone) is known to disrupt the attraction of male insects to the sex pheromones produced by females of a number of insect species. Such compounds may offer potential as control agents. Among the Lepidoptera, such disruption has been reported for Trichoplusia ni (Hübner) (Tumlinson et al., 1972), Lymantria dispar (L.) (Cardé et al., 1973), Grapholitha molesta (Busck) (Gentey et al., 1975; Rothschild, 1975, 1979; Cardé et al 1977b; Cardé, 1981), Argyrotaenia velutinana (Walker) (Cardé et al., 1975; Roelofs, 1978), Choristoneura rosaceana (Harris) (Novak et al., 1978), Choristoneura fumiferana (Clem.) (Sanders, 1975, 1979; Sanders et al., 1982), and Laspeyresia pomonella (L.) (Roelofs et al., 1972; Cardé et al., 1977).

The discovery of a female sex pheromone in the California red scale, Aonidiella aurantii has been reported (Tashiro and Chambers, 1967), and recently, the sex pheromone was indentified by Roelofs et al., (1977).

Since the first application of this method to the pink bollworm moth, Pectinophora gossypiella (Saunders), several other insect pests have been controlled in different parts of the world. The disruption of pheromone communication method, integrated with other control systems, could be applied more usefully for the control of many more insect pests.

Theoretically, disruption of pheromone communication is applicable to all sexually reproducing species, but its application requires a sound knowledge of the ecology, behaviour and population dynamics of the candidate species.

In view of the paucity of information in this area, and the probable importance of the influence of temperature, humidity and light intensity on male emergence, longevity, and the occurrence of mating was investigated. We also wished to determine the optimal placement of traps in the environment to obtain maximum captures of males, or of how environmental and seasonal variables influence trap captures. To observe the behaviour of flying insects under as nearly natural conditions as possible, a trap was designed to test the responses of flying males to sex pheromone released by virgin females in a large corridor used as wind tunnel.

This thesis is concerned with the biology, and certain aspects of intraspecific behaviour in A. aurantii.

The specific objectives were:

a) to estimate the effect of various environmental factors on the mating behaviour of males;

b) development of methods of assaying sex-pheromone and determination of the behaviour of males under the influence of

female pheromone;

c) to study the techniques of marking males and observing the pheromone-searching behaviour in a wind tunnel;

d) to examine the effect of wind velocity and direction on pheromone trail-following by flying males in a wind tunnel;

e) to determine the effect of various environmental factors on attraction of males to traps baited with virgin females;

f) to investigate the possible disruption of pheromone communication with synthetic pheromone.

CHAPTER 2

STUDIES ON THE BIOLOGY OF RED SCALE USING LEMON FRUIT AND LEAF DISCS

2.1 Introduction

The biology of California red scale, Aonidiella aurantii has been studied by a number of workers (Quayle, 1911; Bliss et al., 1931; Nel, 1933; Jones, 1936; Dickson and Lindgren, 1947; Munger and Cressman, 1948; Bodenheimer, 1951; Ebeling, 1959; Tashiro and Beavers, 1968). The species has a diaspine life cycle which Ebeling (1959) synoptically presented as follows: Male-(a) crawler, (b) 1st-instar settled stage, (c) 2nd-instar (nymph), (d) 3rd-instar (prepupa), (e) 4th-instar (pupa), and (f) adult; Female-(a) crawler, (b) 1st-instar settled stage, (c) 2nd-instar, and (d) 3rd-instar (adult).

A comparison of data from studies in California, Zimbabwe and the Middle East shows remarkable differences in the developmental times of any particular stage at a given temperature. These discrepancies can probably be accounted for mainly by differences in host plants and different criteria for separating the instars rather than differences between the biology of red scale itself in the various countries.

However, to facilitate studies on the potential use of the insect's female sex pheromone for control, more detailed information on the influence of environmental conditions on development, longevity and frequency of copulation on different host plants are needed.

2.2 Leaf discs and lemon fruits and their application to the present study

2.2.1 Discussion of methods for rearing red scale

Since red scale is able to develop on a large number of plant

hosts (Quayle, 1938b; Bodenheimer, 1951), many different rearing methods have been tried. A satisfactory host plant for experimental purposes is one that is readily available and possesses a form and habit that permits ease in handling, the efficient transfer of infestation, and furnishes a large area of infestation per unit of space occupied by the host plant (Flanders, 1951). The host plants of red scale that have been found to meet these requirements are lemon leaves and fruits.

For rearing large numbers of scale, as distinct from rearing for detailed observation, Flanders (1951) and Debach and White (1960) tested several hosts including: banana squash (Cucurbita maxima), citron (Citrullus vulgaris), butternut squash (Cucurbita moschata), potatoes, grapefruit and lemon. For continuous mass rearing of red scale at low cost they found that banana squash was the best host.

In the present study banana squash was used to maintain a stock culture of red scale for use in infesting lemon leaves and fruits.

Four methods are commonly used for transferring red scale to fresh host plants: (1) the contact method; which involves the temporary placement of the fresh host plant on top of the mother infestation so that the crawlers can move from one to the other; (2) the drop method; which involves placing fresh host plants beneath the mother infestation, the crawlers dropping from the latter when unable to maintain a foothold because of crowding; (3) the brush method; which involves brushing the crawlers from the mother infestation with a soft camel's hair brush; and (4) the blowing method; which involves the use of compressed air (Flanders, 1951).

The brush method for transferring scale was found to be most convenient and efficient, especially when transferring crawlers to small host plants, like lemon leaf discs.

2.2.2 The leaf disc method

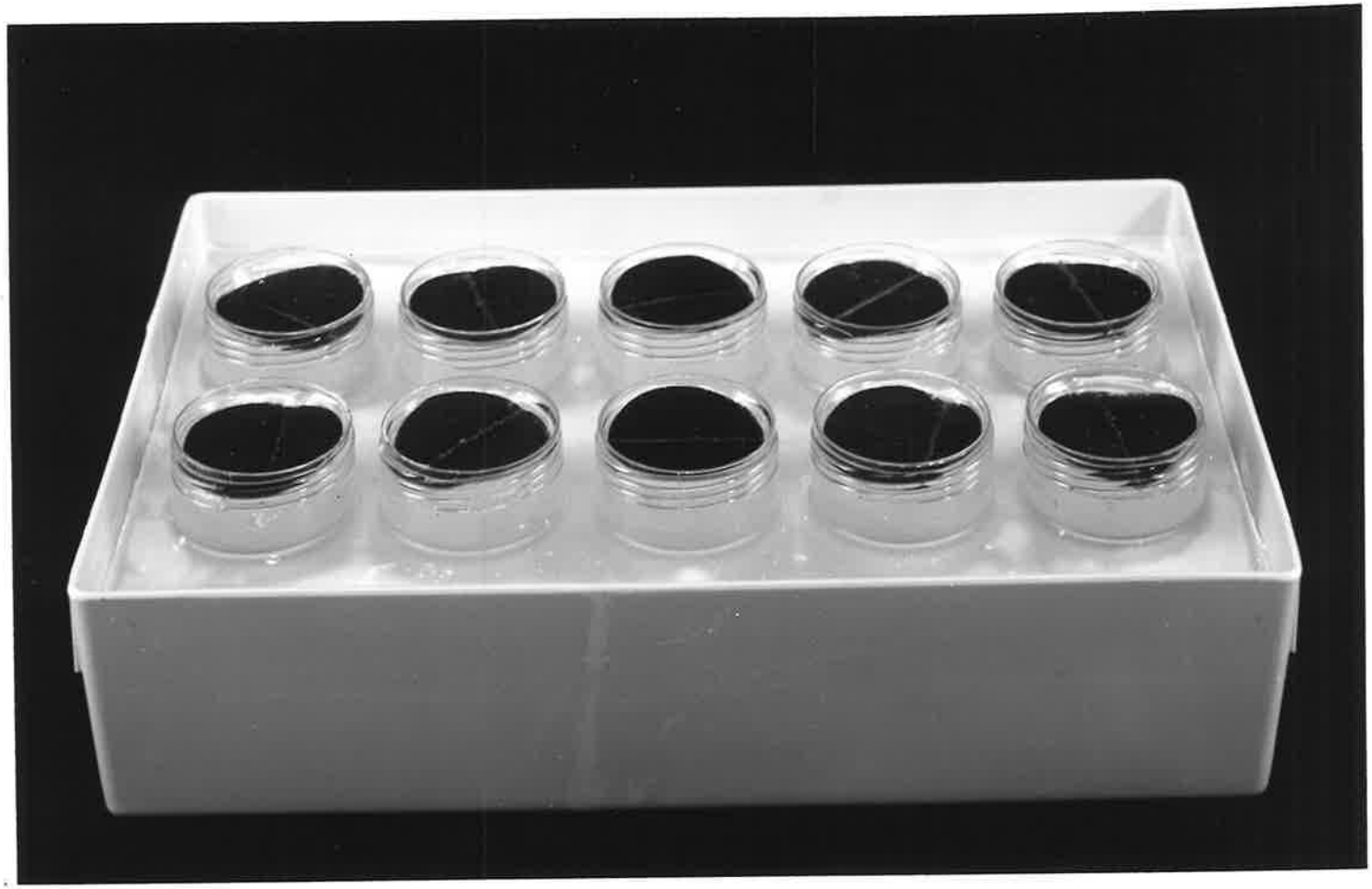
Willard (1968) showed that discs cut from citrus leaves were a convenient method of rearing red scale, and this method was used as a standard.

Discs were cut from lemon leaves using a 30 mm. diameter cork borer and immediately floated on distilled water in 50 x 35 mm plastic vials. Red scale crawlers were transferred to the leaf discs with a fine brush. Usually 85% or more of these crawlers settled on the discs, while the remainder either died before settling or were trapped in the water at the edge of the disc. If the disc was cut with the main vein through the centre, most of the crawlers settled close to this vein and away from the edge of the disc where the chance of drowning was much greater (Willard, 1968). Generally discs with the main vein were flatter, had less tendency to curl at the edges, and floated evenly on the surface of the water. Tests using small numbers of discs suggested that the longevity of discs with or without the main vein was similar. Scales developed and reproduced normally on leaf discs. Frequently two or more generations of scale could be reared on the same disc.

The use of leaf discs had several advantages over other methods of rearing red scale (Willard, 1968):

(1) Economy of space: A paraffin tray of 10 vials gave 10 separate plots in an area of 150 x 225 mm (Fig. 2.1).

Fig. 2.1 Paraffin tray to hold ten 50 x 35 mm plastic vials. Lemon leaf discs with populations of red scale.



(2) Uniformity: By careful selection of leaves, it was possible to obtain discs of uniform texture and colour. When the discs were in one plane it was possible to expose all discs to uniform temperature and light intensity.

(3) Ease of observation: The entire area of a disc could be inspected at once under the 6X of a binocular microscope without altering the focus. For this reason it was possible to locate the scale immediately and make the necessary observations without loss of time in searching for the insect.

(4) Water Barrier: The water around the edge of the disc formed an effective barrier to the red scale crawlers with very low losses due to drowning.

The leaf disc is an artificial environment for the scales, and consequently the interpretation of experimental results must be made with caution. In general, the leaf disc method was found to be quite satisfactory for rearing and studying red scale in the laboratory.

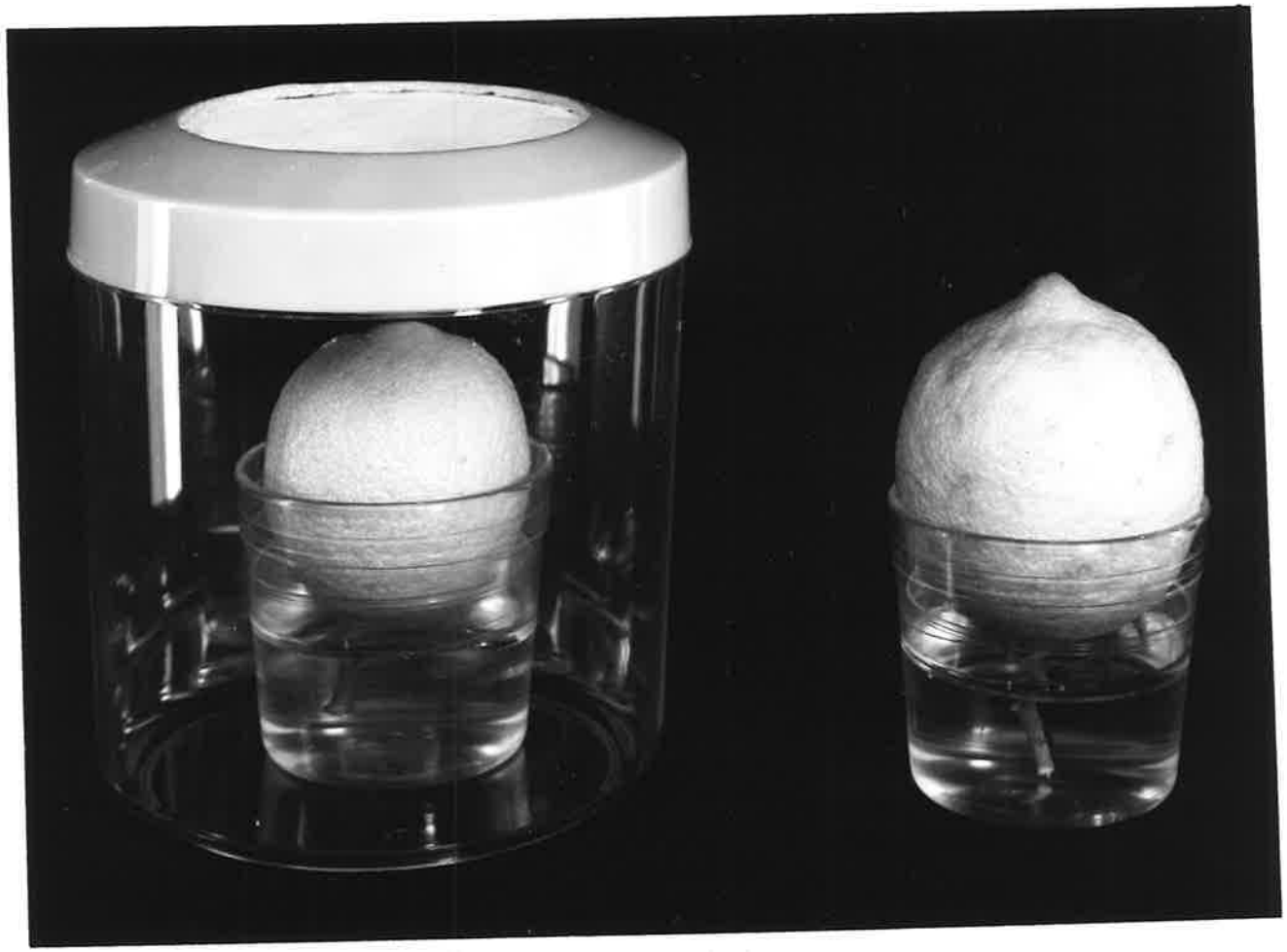
2.2.3 The lemon fruit method

Lemons (coated with paraffin on the stem end) were found to be the most convenient host for rearing large numbers of crawlers for laboratory experiments (Debach and Sundby, 1963).

Green turgid fruit with 3-4 cm stems were infested with crawlers and maintained on wide-mouth plastic vials filled with distilled water in which the stems were submerged(Fig. 2.2).

Scales developed and reproduced normally on lemon fruit. Frequently three or more generations of scale could be reared on the same fruit.

Fig. 2.2 Green turgid fruit with 3-4 cm stems were maintained on wide-mouth plastic vials filled with distilled water (right) and held in hostess plastic canister (left) when the sexes became distinguishable.



During the present study, the lemon fruits with stems in the water were found to be convenient and efficient for laboratory and field experiments. Particularly, virgin females reared on lemon fruit and placed in the sticky slide trap were used for studies on the flight of males attracted to female pheromone in the wind tunnel and the field trapping tests.

2.3 Development of red scale reared on leaf discs

According to Tashiro and Beavers (1968) reports on the life cycle of red scale on lemon fruits, an experiment was performed to study the development of red scale on lemon leaf discs.

Leaves were taken from one lemon tree and 10 leaf discs cut immediately. Each disc was then floated on distilled water in plastic vials, and 80 crawlers were transferred to each disc on the same morning, the discs were kept under a bank of white fluorescent light producing an intensity of 200 lm./ft.^{2} and 10L : 14D beginning at 09.00 h; the laboratory was maintained at 25 ± 0.5°C and an ambient relative humidity of 75 ± 5% RH. At intervals varying from daily to semi-weekly. depending on the rapidity of scale development, 5 specimens were removed from each disc, and their developmental stages were recorded. When the sexes became distinguishable, 5 specimens of each sex were removed from each dsic during each examination. Table 2.1 summarizes the information obtained.

The crawlers wander for a short time after transfer, usually only a few hours, then insert their stylets into the plant tissue and begin feeding, and become white caps within 24 h.

Within a day of settling the white cap begins to darken and become more compact. As the insect develops it extends the wax along the surface of the host in a circle around the white cap (the "nipple stage") (Fig. 2.3).

Table 2.1 Growth and development of the California red scale at 25 ± 0.5°C and 70 ± 5% RH.

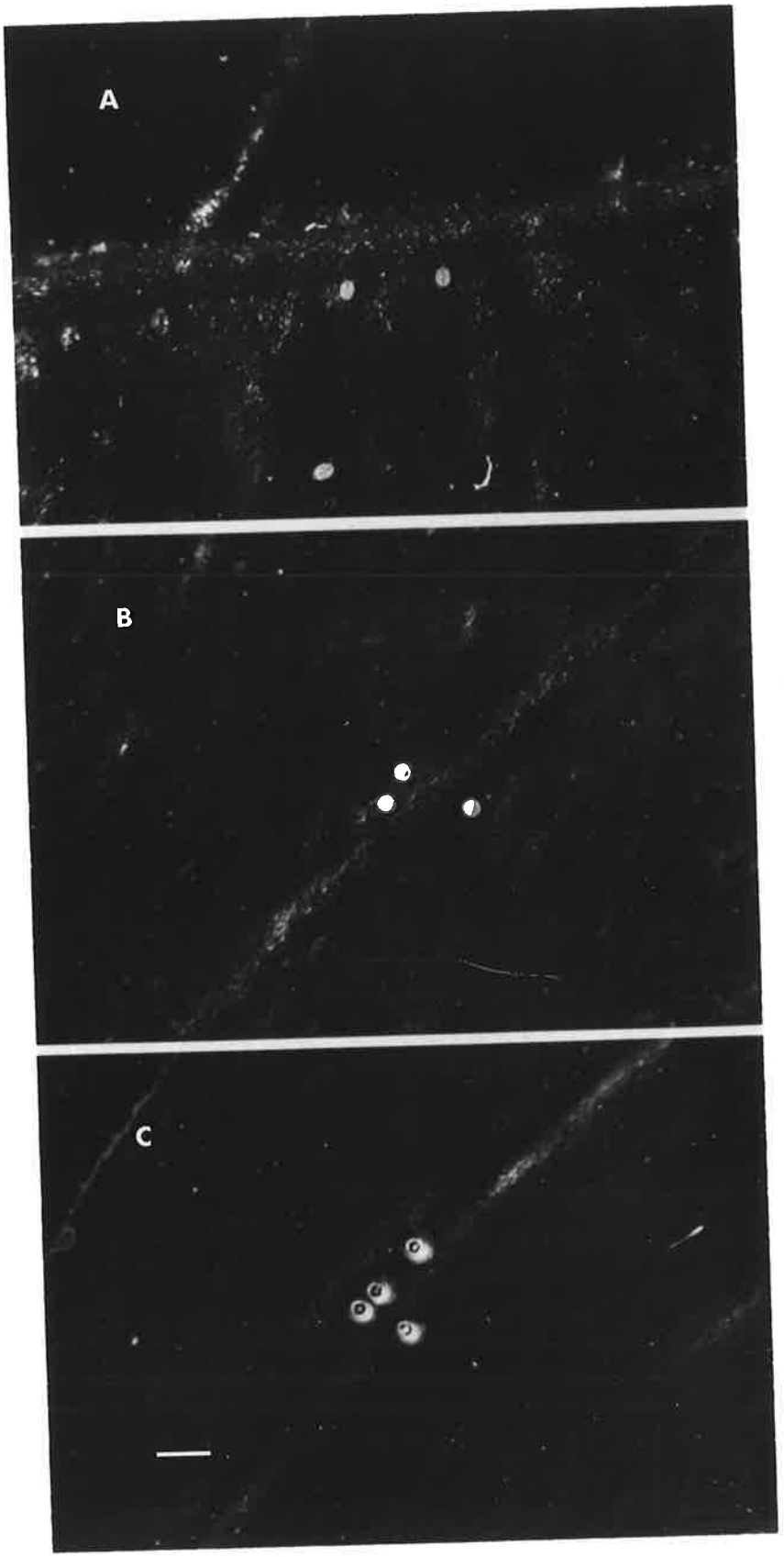
Stage	Number of days from crawlers to Beginning of stage	Peak of stage	Beginning of stage	Stage
Males and Females:				
White cap (1st instar)	1	1		
Nipple (1st instar)	2	3		
First molt (to 2nd instar)	10	10		
Gross sex differentiation first evident	16	18		
Gross sex differentiation evident in 98% of specimens	18	18		
Males only:				Females only:
Nymph (2nd instar)	16	18	16	Female (2nd instar) occupying 1st molt gray margin
Second molt, to prepupae (3rd instar)	19	20	19	Second molt (3rd instar) cover tight
Third molt, to pupae (4th instar)	24	25	24	Molt complete, cover loose
Fourth molt, to pre-emergence adults (4th instar)	26	27	26	Gray margin extending
Adult emergence	26	29		
		28	27	Gray adult, pygidium extended
		31	31	Inseminated, pygidium retracted
		40	40	Embryonic formation visible
		47	46	Crawlers under scale and emergence

Fig. 2.3 A: Emergence of crawlers on a lemon leaf disc.

B: Crawlers that had just settled and formed a "white cap".

C: Within a day of settling the white cap begins to darken and become more compact "Nipple stage".

Scale represents 0.25 mm.


A
B
C

Growth and development were gradual until the sexes could be distinguished without removal of the scale covering. Males were first distinguishable, at 16 days past the crawler stage, by the elongation of the scale covering and the appearance of the eyes, which may be obscure or plainly visible through the scale covering; the sex of almost all specimens was apparent by the 18th day (Fig. 2.4). After the second moult, the male enters the prepupal stage coinciding with the 2nd (and last) molt of the females. When the sheaths of the antennae and wings are visible males become pupae (3rd molt) during the completion of the 2nd molt of the females. A fourth moult brings the male to maturity, and a short time after this final moult the male pushes its way backward under the waxy scale and emerges as an alate adult (during transformation of the males to adults, the gray margin of the adult female rapidly extended). The 1st adult males were present within 26 days, this timing coincided with a rapid extension of the gray margin of females, indicating mating receptivity (Fig. 2.5). If fertilization occurs, the female body greatly increases in size and extends to the edge of the scale; the colour of the body changes from yellow to red-brown. The crawlers began to emerge on the 46th day after insemination.

2.4 Comparison of the development of red scale reared on leaf discs and lemon fruits

Lemon leaf discs and fruits were used as hosts for rearing and maintaining scales in many of the experiments in this study. In order to evaluate and compare the two methods of rearing scales, the development of red scale on lemon leaf discs and fruits was evaluated at four temperatures (15, 20, 25 and 30°C).

Leaves were taken from one lemon tree and 40 leaf discs cut immediately,

Fig. 2.4 Males (2nd instar, nymph) were first distinguishable by the elongation of the scale covering. The females scale covering were nearly circular. Scale represents 0.8 mm.

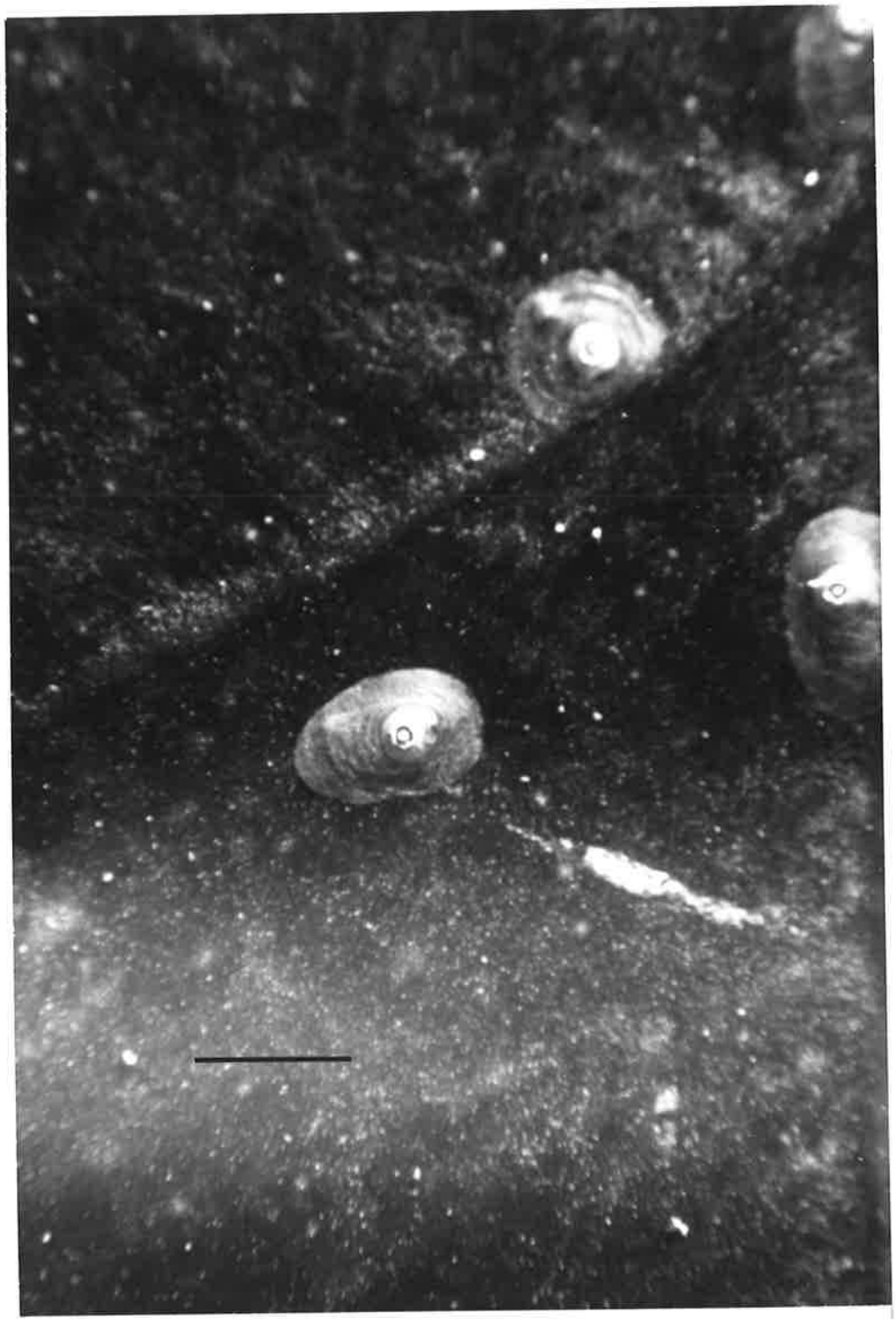
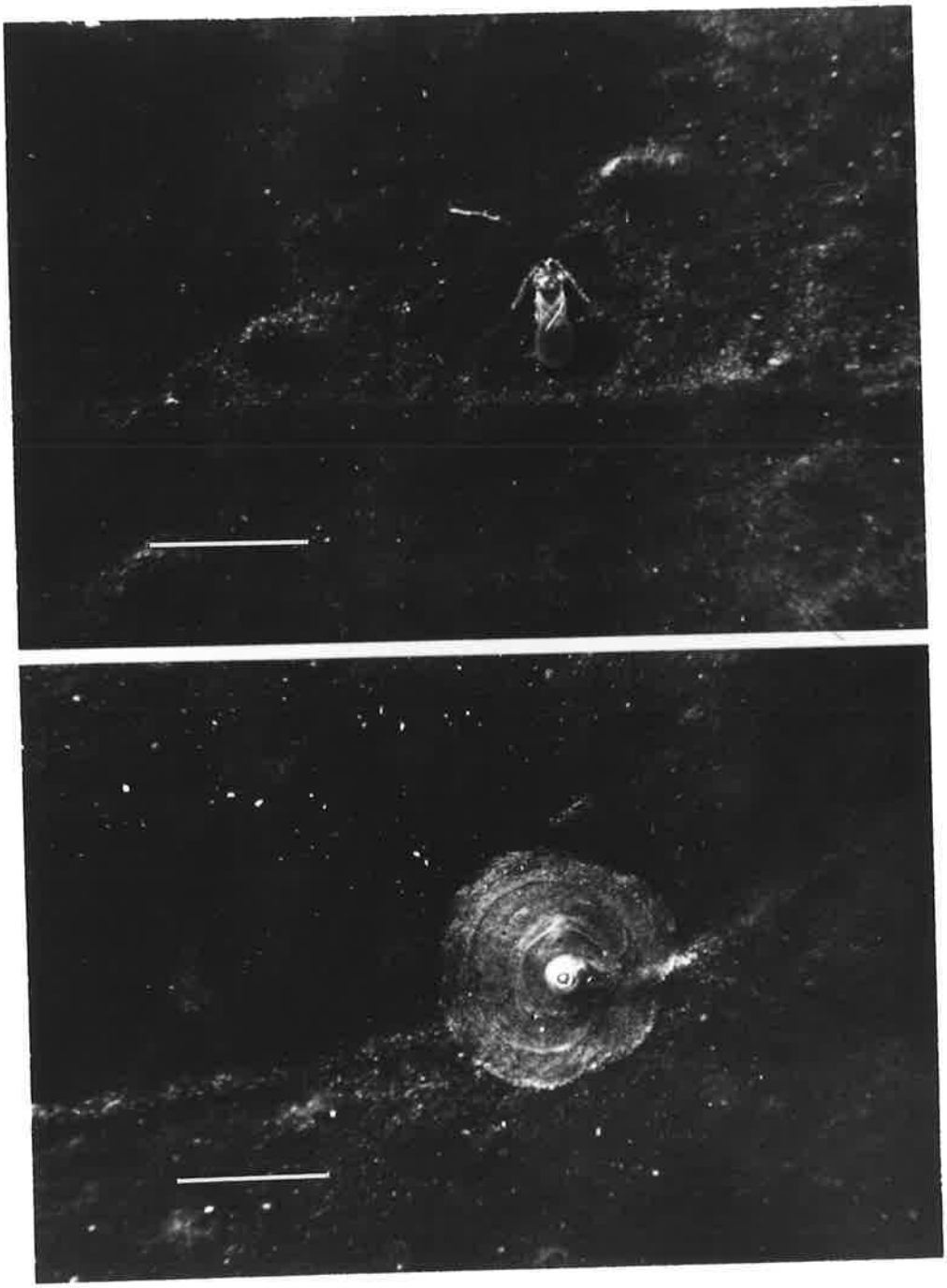


Fig. 2.5 Top: Adult male.

Bottom: Adult female with a rapid extension of the gray margin indicating mating receptivity.

Scale represent 0.8 mm (top) and 1.1 mm (bottom).



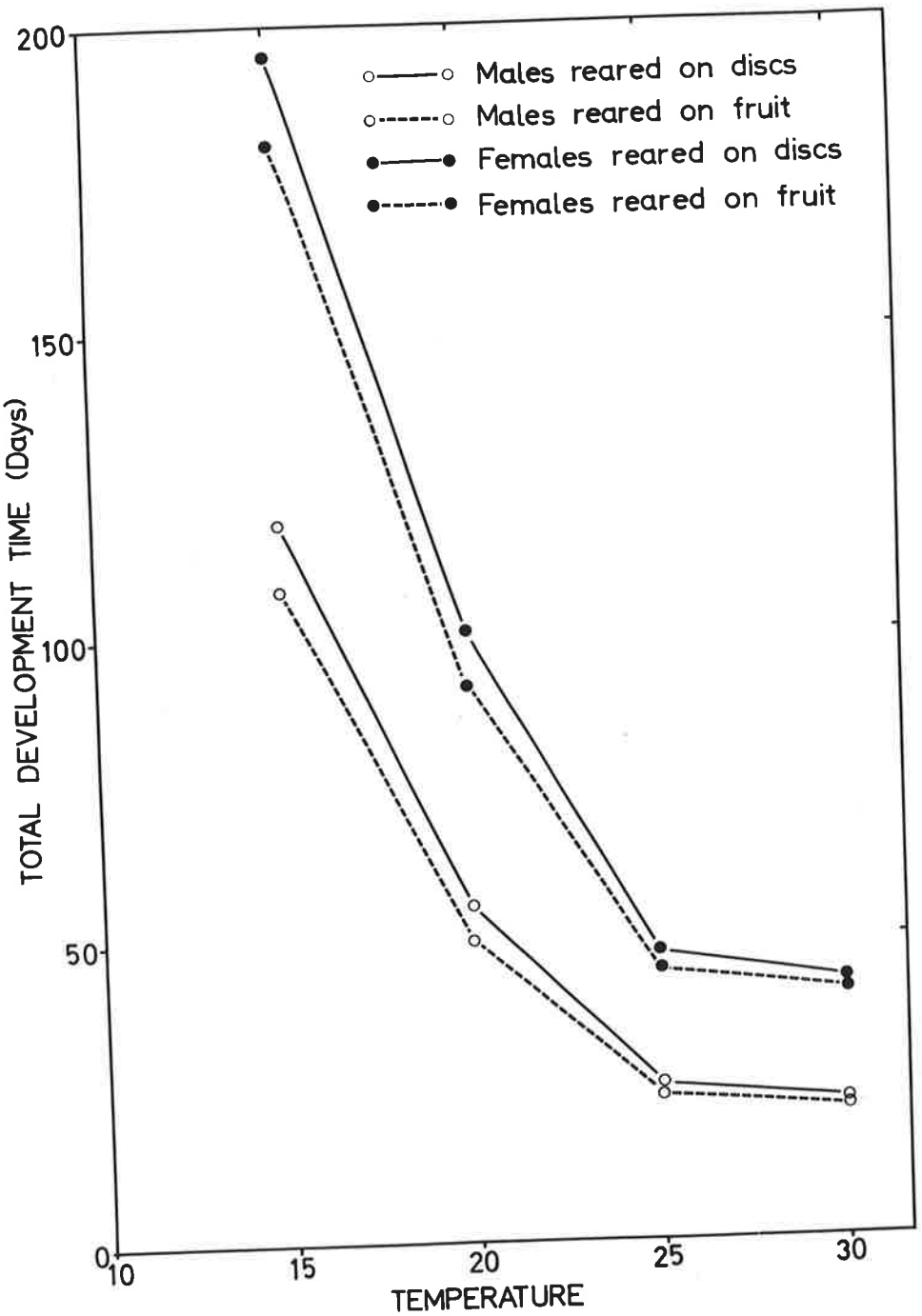
then randomized into four groups. Eighty crawlers were transferred to each disc on the same morning, and the discs immediately moved to the required temperatures. At the same time, 40 lemon fruits were infested with crawlers and moved to required temperatures. The discs and fruits were kept in various temperature rooms under white fluorescent lights at 200 lm./ft.^{2} and a 10L : 14D photoperiod beginning 09.00h.

Observations were made at 25 and 30°C daily or twice a week at 15 and 20°C, 5 specimens were removed from each disc and fruit, and their developmental stages were recorded until the males emerged or the females had completed the third instar and had reached the "gray adult", they were exposed to a number of males to allow fertilization to take place. The females reared at 15°C were moved to 25°C for fertilization since the males were not active at 15°C. The total duration of development from crawler to crawler in the female; total duration of development of the male from crawler to emergence of the adult at each temperature is shown in Fig. 2.6.

2.5 Seasonal occurrence

Red scale passes through two to three generations a year in South Australia (Furness, 1973). Emergence of crawlers commences in late September and continues until about mid-November. From emergence of crawlers to the next generation of crawlers takes about 3 months. The development of the second summer generation takes longer, and crawler emergence to females producing crawlers takes about 3-4 months. The earlier developed adults of the second generation may either produce a third generation that will over-winter as larvae and pupae, or adults. The later developed of the second generation may over-winter in the host, on which they develop through winter.

Fig. 2.6 The duration of total development of red scale males and females reared on lemon leaf discs and fruits in relation to temperature.


Males reared on discs
Males reared on fruit
Females reared on discs
Females reared on fruit
TOTAL DEVELOPMENT TIME (Days)
200
150
100
50
0
10
15
20
25
30
TEMPERATURE

CHAPTER 3

STUDIES ON MALE EMERGENCE AND MATING BEHAVIOUR OF CALIFORNIA RED SCALE

3.1 The emergence of males

3.1.1. Introduction

Males are first distinguishable at 16 days past the crawler stage, by the elongation of the scale covering and by the appearance of the eyes, which may be plainly or obscurely visible through the scale covering.

During the earlier part of the pupal stage, the joints of the appendages become clear-cut; the lighter divisions marking the segments are not clearly seen until shortly before the final moult. The bristles on the legs and antennae are difficult to detect at first, but become clearer and better defined with the formation of the final cuticle. The evaginated style and all the other appendages are clearly seen enclosed within their thin, cuticular sheaths; the wings are collapsed and folded within their sacs. The eyes change from diffuse areas to the definite areas that they occupy in the adult male. The last or final moult produces the fully developed male, which remains under the shelter of its scale covering for a day or so before emerging (Nel, 1933).

Bodenheimer (1951) stated that the males were nocturnal. However, Nel (1933) stated that males emerged in the morning. Tashiro (1968) found that most of the males emerged and were in flight in late afternoon. Observations made in our rearing room indicated that most of the males emerged in late afternoon. Emergence began at 15.00 h, reched its peak between 17.30 and 18.30 h and had practically ceased at 20.00 h. In the

morning and at night very few males emerged. Similarly, in the field, large numbers of active males were observed in the late afternoon and very few in the morning or at night.

Observation indicated that almost all the males emerging during a given afternoon were dead by the next morning. However, Nel (1933) reported emergence at 09.45 h and death by 10.35 h. Quayle (1911) stated that adult males lived from 1 to 5 days. Tashiro et al. (1968) reported that mean longevity of males was 6-7 h. It was observed that mortality commenced 3 h after emergence, about half the males were dead within 5-6 h. And all were dead within 12 h in the 25°C rearing room.

These observations suggested that there was a diel rhythm of emergence possibly circadian in nature and that the rhythm might be controlled by temperature and light. A series of experiments was carried out to determine whether such a rhythm did exist, as well as the environmental factors involved the emergence of males. The experiments were also designed to determine the longevity of males.

3.1.2 Materials and Methods

Leaves were taken from lemon trees and one disc (30 mm in diameter) was cut from each leaf. Fifty crawlers were transferred to each disc the same morning, all discs were then kept at 25°C under a bank of white fluorescent lights giving 400 lm./ft.^{2} at the level of the discs. A 12 h photoperiod from 08.00 to 20.00 h followed by 12 h darkness (12L:12D) was used to correspond approximately to the conditions in the field at the time the leaves were picked.

After 16 days, when the sexes become distinguishable, 20 males

were selected from each disc and all others removed. During the pre-emergence adult stage, the discs were moved to the required treatments. A treatment consisted of 100 males and observations were made 4 consecutive days. As soon as emergence began, the number of males on each disc was counted every 15 or 30 min until dark, and a final observation was made the next morning (07.00 h) to determine emergence during the night. For each 15 min or half-hour interval the total number of males emerging from 100 pre-emergence adults was obtained.

3.1.3 Time of the day of male emergence

Adult eclosion in many insect species typically has a distinct did pattern which tends to be correlated with the rhythms of locomotor activity and reproductive behaviour. In the diurnal dragonfly, Tetragoneuria cynosura, studied by Lutz (1961), 75% of the adult forms emerging on a given day did so before 9.00 h. Callahan (1958) observed that 95% of the adult of the corn earworm, Heliothis zea, emerged between 19.00 and 23.00 h; this species displays a nocturnal flght habit. A number of Chironomidae species were found to emerge principally between sunset and midnight, with the maximum numbers emerging near midnight (Palmen, 1955). Many species of small diurnal Diptera have been found to display morning maxima of adult emergence: Drosophila (Bünning, 1935), Dacus tryoni (Myers, 1952), Scataphage stercoraris (Lewis and Bletchly, 1943), Pegomyia betae (Dunnung, 1956). These are sufficient to illustrate the point that adult emergence is not randomly distributed through the diel cycle, but frequently displays a high degree of specialization (Beck, 1980).

Under natural field conditions many environmental factors might be

expected to influence the emergence of adult insects. It is also conceivable that different species might have evolved the ability to utilize different natural variable as signals in adjusting their times of emergence. The diel cycles of temperature, humidity, and light intensity might well be involved (Beck, 1980).

Experiment were conducted to observe the male emergence in the laboratory and field conditions.

Experiment 1 :

All scales were reared on lemon leaf discs (20 scales per disc) and held in the laboratory at 25°C, 70±5% ambient RH and a level of illumination of 400 lm./ft.^{2}. Observation began at 08.00 h and the number of males on each disc was counted every 30 min until 20.00 h. Emergence began at 15.00 h, reach its peak between 17.30 and 18.30 h, and practically ceased at 20.00 h (Table 3.1).

Experiment 2:

During mid-November, lemon leaf discs bearing adult males were placed outdoors in the afternoon and exposed to the sky. A photometer (Eel Model 18/2169, Eel International Ltd., Australia) and Wallac thermometer (Manufactured by Wallac Oy Co. Ltd., Finland) was used to measure the levels of illumination and temperature. As soon as emergence began, the number of males on each disc was counted every 30 min until dark, and a final observation was made the next morning (07.00 h) to determine emergence during the night.

Emergence of 82 males was recorded, the emergence began at 14.30 h, and peak emergence occurred between 17.00 and 18.30 h, and then decreased

Table 3.1 Time of day and indoor emergence of males of California red scale reared on lemon leaf discs in the laboratory at 25°C and 70 ± 5% ambient RH.

Hour counted	Number of males emerging					Total
	Disc 1	Disc 2	Disc 3	Disc 4	Disc 5	
14.30	0	0	0	0	0	0
15.00	0	1	0	0	0	1
15.30	0	0	0	0	1	1
16.00	0	0	0	1	1	2
16.30	1	1	2	1	0	5
17.00	2	2	1	1	1	7
17.30	4	4	3	2	3	16
18.00	4	3	6	4	3	20
18.30	2	1	2	4	5	14
19.00	0	3	1	1	3	8
19.30	1	1	1	0	0	3
20.00	0	0	1	1	0	2
Dark	0	0	0	0	0	0
Total	14	16	17	15	17	79

rapidly after 19.30 h (Table 3.2). The results of male emergence in the field indicated that the emergence occurred in the afternoon, but half an hour earlier than in the laboratory (Fig. 3.1).

3.1.4 Effect of temperature on male emergence

An experiment was carried out to determine the effect of temperature on male emergence. All scales were allowed to develop to the final moult stage and then were moved to the experimental temperatures (15, 20, 25, 30 and 35°C) and exposed to 12L:12D, the numbers emerging every 30 min were recorded. The results are given in Table 3.3.

From Table 3.3 it can be seen that with an increase of temperature the distribution of emergence was shifted closer to midday, i.e. the peak emergence at higher temperature occurred earlier than at lower. The numbers of emergence were similar for 35°, 30° and 25°C, but for the lower temperature of 15°C, only 58% of males emerged.

A temperature of 40°C was also tested, but in this circumstance only 15% of the males emerged. The males are inactive at such temperature.

3.1.5 Effect of relative humidity on male emergence

An experiment was set up to determine whether difference in relative humidity in the constant temperature rooms were influencing the time of male emergence. Four levels of relative humidity were tested (30-35%, 40-45%, 60-65%, 80-85%), and since all four humidities could not be maintained simultaneously, each treatment was done separately.

All scales were held in the laboratory at 25°C and 70±5% ambient RH. During the pre-emergence stage, the leaf discs were placed in a plastic petri dish, and the dishes were moved to the experimental ambient humidities. Humidity was controlled by a Defensor 505 humidifer coupled with a hygrostat

Table 3.2 Time of day and outdoor emergence of male California red scale reared on lemon leaf discs.

Hour[a] counted	Number of males emerging					Total	Levels of illumination (lm./ft.^{2})	Mean temperature (°C)
	Disc 1	Disc 2	Disc 3	Disc 4	Disc 5			
14.00	0	0	0	0	0	0	865	29
14.30	0	0	1	0	0	1	790	29
15.00	0	1	0	0	1	2	715	28
15.30	1	0	0	1	0	2	650	28
16.00	1	2	0	0	1	4	620	28
16.30	2	1	1	1	1	6	580	27
17.00	4	2	4	3	2	15	510	26
17.30	3	2	5	4	4	18	440	26
18.00	2	3	3	3	2	13	360	25
18.30	2	2	1	3	3	11	325	25
19.00	1	1	1	2	1	6	190	23
19.30	0	1	0	1	1	3	65	21
20.00	1	0	0	0	0	1	< 1	19
Night	0	0	0	0	0	0	>0.1	15
Total	17	15	16	18	16	82		

a) Pacific daylight time.

Fig. 3.1 Emergence of males red scale under laboratory and field conditions.

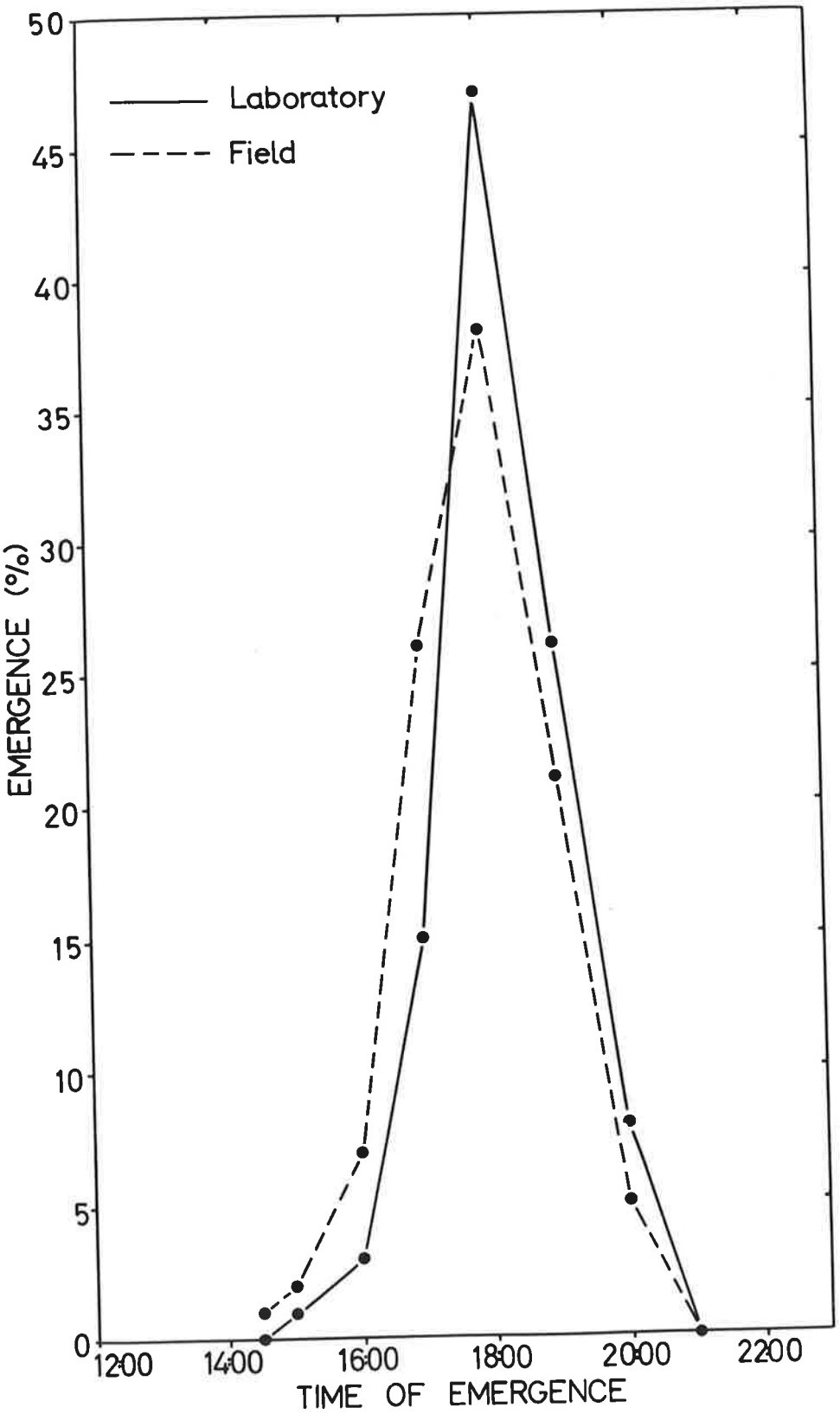

Laboratory
Field
EMERGENCE (%)
50
45
40
35
30
25
20
15
10
5
0
1200
1400
1600
1800
2000
2200
TIME OF EMERGENCE

Table 3.3 Effect of various temperatures on the emergence of California red scale. (At 70 ± 5% ambient RH).

Hour counted	Number of males emerging at -				
	35°	30°	25°	20°	15°
14.00	0	0	0	0	0
14.30	1	1	0	0	0
15.00	3	2	1	0	0
15.30	3	3	0	1	0
16.00	8	4	3	1	0
16.30	27	6	5	3	1
17.00	20	15	10	3	2
17.30	9	26	17	8	4
18.00	6	12	23	15	5
18.30	3	4	8	21	18
19.00	3	5	5	12	15
19.30	2	3	6	7	6
20.00	0	0	2	5	7
20.30	0	0	0	0	0
Total	85	81	80	76	58

range from 30 to 85% RH. The results are given in Table 3.4.

The beginning of the emergence times and the peak emerging period were similar to 40-45% RH and 60-65% RH, but at 30-35% and 80-85% RH emergence was delayed. Table 3.3 and Table 3.4 shows the effects of the laboratory environment on red scale males emergence. A temperature of 25°C and 70±5% RH was near optimum for male emergence in the laboratory.

3.1.6 Effect of light intensity on male emergence

Harker (1964) reported that some animals respond to a change of light intensity from low to high in the same way that they respond to a change from dark to light.

An experiment was conducted to estimate the effect of light intensity on male emergence at eight light intensities ranging from 25 to 1000 lm./ft.^{2}. In this experiment all treatments were giving a preliminary period of 12 h of complete darkness. intensities of 25, 50, 100, 200, 400, 600, 800 and 1000 lm./ft.^{2} were obtained by decreasing the vertical distance of the leaf discs from the center of a bank of fluorescent tubes. Vials were placed in black trays to minimize reflected light.

The photoperiod was 12 h from 08.00 h and observations were made at half hourly intervals from 08.00 to 20.00 h. The results are given in Table 3.5.

The pattern of emergence was similar for 1000, 800, 600, 400 and 200 lm./ft.^{2} intensities, and the times of peak emergence were nearly the same, but emergence was delayed at 50 and 25 lm./ft.^{2}.

The second experiment was designed to estimate the threshold light

Table 3.4 The effect of relative humidity on the emergence of California red scale males at 25°.

Hour counted	Number of males emerging at an ambient RH of -			
	30-35%	40-45%	60-65%	80-85%
14.30	0	0	0	0
15.00	0	1	2	0
15.30	1	3	2	0
16.00	3	4	5	1
16.30	2	6	9	3
17.00	5	10	12	4
17.30	6	14	20	6
18.00	10	18	16	9
18.30	17	9	4	16
19.00	14	6	6	13
19.30	7	2	4	6
20.00	4	3	2	7
20.30	0	0	0	0
Total	69	76	82	65

Table 3.5 The effect of eight light intensities on the emergence of California red scale males at 25°C.

Hour counted	Number of males emerging at 1m./ft.^{2} of -								
	1000	800	600	400	200	100	50	25	Dark
14.30	0	0	0	0	0	0	0	0	0
15.00	3	1	2	0	0	0	0	0	0
15.30	2	4	3	2	2	1	0	0	0
16.00	4	6	7	3	2	1	1	0	0
16.30	7	4	6	7	3	2	1	0	0
17.00	15	11	20	9	6	4	4	2	0
17.30	23	21	15	19	11	9	5	3	0
18.00	9	16	12	21	15	18	7	6	0
18.30	8	6	6	11	17	13	8	5	0
19.00	4	6	5	9	7	10	18	13	0
19.30	2	4	4	3	5	7	11	10	0
20.00	0	1	1	0	2	4	5	4	0
20.30	0	0	0	0	0	0	0	0	0
Total	80	77	81	84	73	67	60	43	0

intensity. Varying light intensities of 400, 25, 8, 4, 2, 1 and 0.5 lm./ft.^{2} and complete darkness were used. The light source was obtained from a small white fluorescent tube operated at low voltage to achieve the required intensities, and differences in intensity from 25 to 1 lm./ft.^{2} were obtained by increasing vertical distance from this source (Lees, 1953a).

All treatments were 12L:12D, light on at 08.00 h. At 17.00 h all treatments were brought to 400 lm./ft.^{2} and observations were made at half hourly intervals to 20.00 h. The experiment was continued over four successive days (Fig. 3.2).

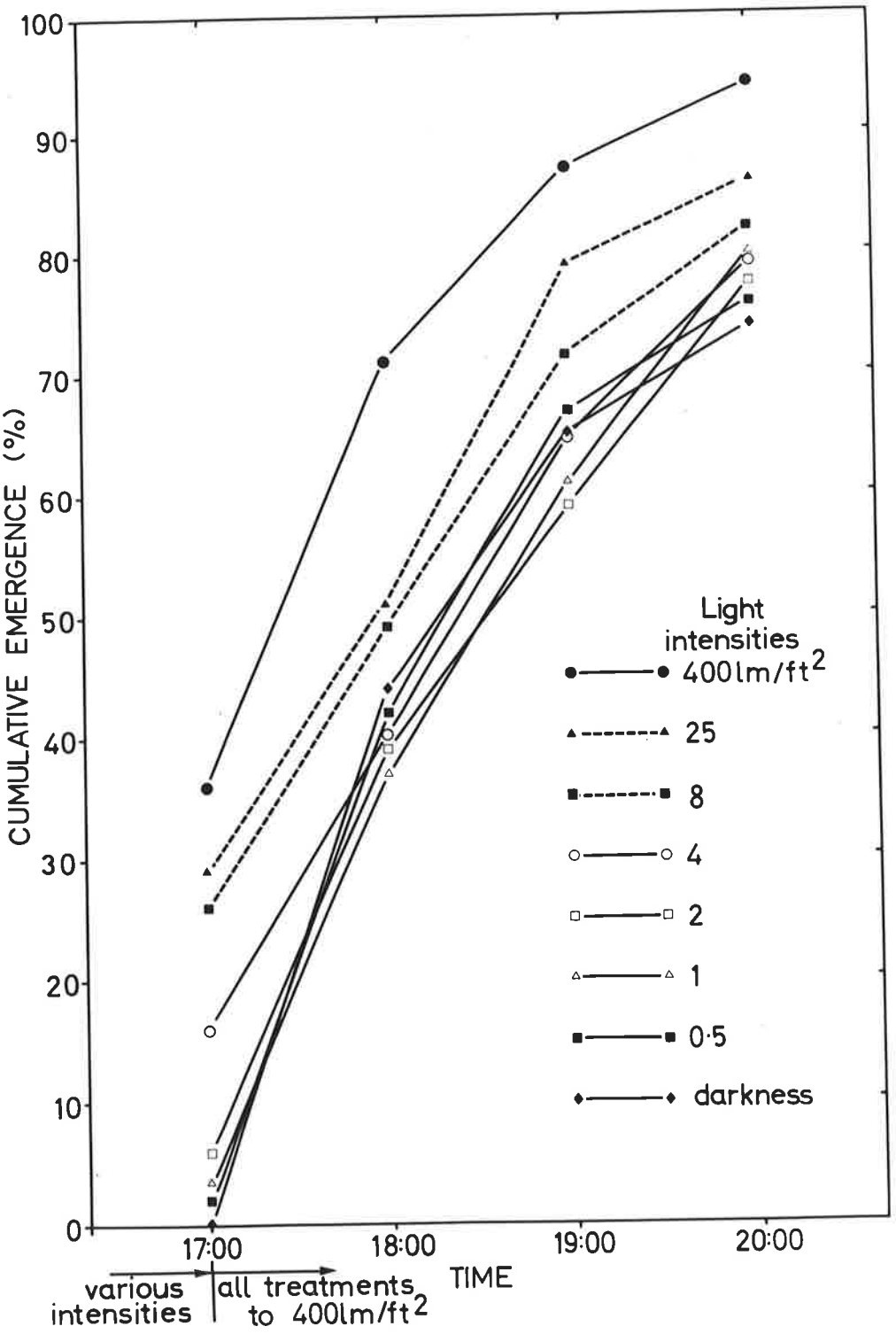
The time of peak emergence was earliest at the highest light intensity tested (400 lm./ft.^{2}) and was delayed by lower light intensities; intensities lower than 2 lm./ft.^{2} gave results similar to complete darkness. These results show that the threshold light intensity probably lies between two and four lm./ft.^{2}. Lees (1955) suggests that the threshold intensity for photoperiodic response in most arthropods is about 1 lm./ft.^{2} but there are also reports of threshold intensities ranging from less than one to three (Dickson, 1949).

3.1.7 Effect of temperature on male longevity

The effect of temperature on the longevity of insects has been widely documented. An inverse relationship between longevity and temperature, within an "optimum" temperature range is commonly acknowledged, although, within the broader temperature range from zero upwards, a bell-shaped relationship has been found (Karandinos and Axtell, 1967).

experiments were carried out to estimate the effect of different constant temperatures on male longevity. Lemons bearing adult males were placed in fiberboard cartons that were balckened inside. Each carton was furnished with a collection chamber consisting of a plastic

Fig. 3.2 Threshold light intensity for emergence-comparison of emergence at six light intensity and darkness in the range 0.5 to 25 lm./ft.^{2}, compared with a control at 400 lm./ft.^{2}.


100
90
80
70
60
50
40
30
20
10
0
CUMULATIVE EMERGENCE (%)
17:00
18:00
19:00
20:00
TIME
various intensities
all treatments to 400lm/ft2
Light intensities
400lm/ft2
25
8
4
2
1
0·5
darkness

tube with one end covered with nylon gauze and its open end was inserted into the lid of the fiberboard container. Males that emerged moved toward the light and were trapped. These males were immediately placed one to a petri dish and held at constand temperatures of 15, 20, 25, 30, 35 and 40°C in groups of 20. Observations were made at hourly intervals until all males were dead.

The results obtained from this experiment indicated that the longevity of males were greatest at the lowest temperature and decreased rapidly with increase in temperature. At the highest temperature of 40°C, mortality commenced 1 h after emergence, about 80% of males were dead within 3 h, and all were dead within 7 h. At the lowest temperature of 15°C, most of the males were inactive, and some lived 29 h.

The longevity of males at different temperatures is given in Table 3.6 and the linear regression of mean longevity on temperature is illustrated in Fig. 3.3.

3.1.8 Effect of relative humidity on male longevity

An experiment was conducted to determine the influence of humidity on male longevity. Emerging males were placed one to a petri dish and held at humidities of 30-35%, 40-45%, 60-65% and 80-85% at 25°C in groups of 20. Observations were made at hourly intervals until all males were dead. The results are given in Table 3.7 and a linear regression of mean longevity on humidity is illustrated in Fig. 3.4.

The results indicated that high humidity favoured the survival of males; some could live for 20 h at 80-85% RH. At the lowest humidity tested, 30-35% RH, mortality commenced at 2 h and about half the males

Table 3.6 Effect of temperature on male longevity of California red scale.

Temp (°C)	n	Longevity (hours) Mean ± S.E.	Range
15	20	17.2 ± 1.3	7-29
20	20	13.5 ± 0.9	5-21
25	20	8.5 ± 0.7	3-16
30	20	7.2 ± 0.7	2-13
35	20	6.3 ± 0.6	2-11
40	20	2.9 ± 0.4	1-7

Fig. 3.3 Male longevity at various constant temperatures.

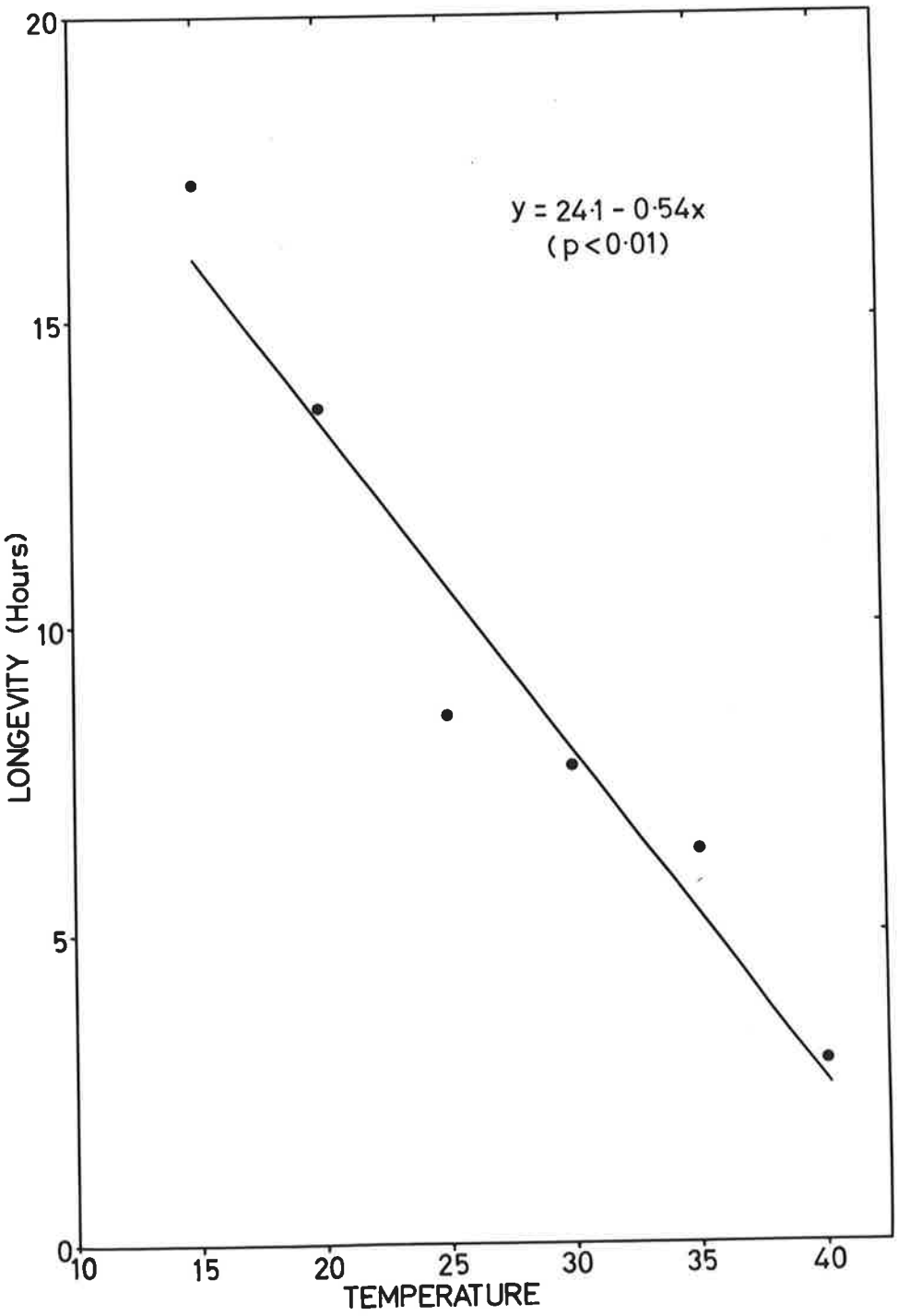
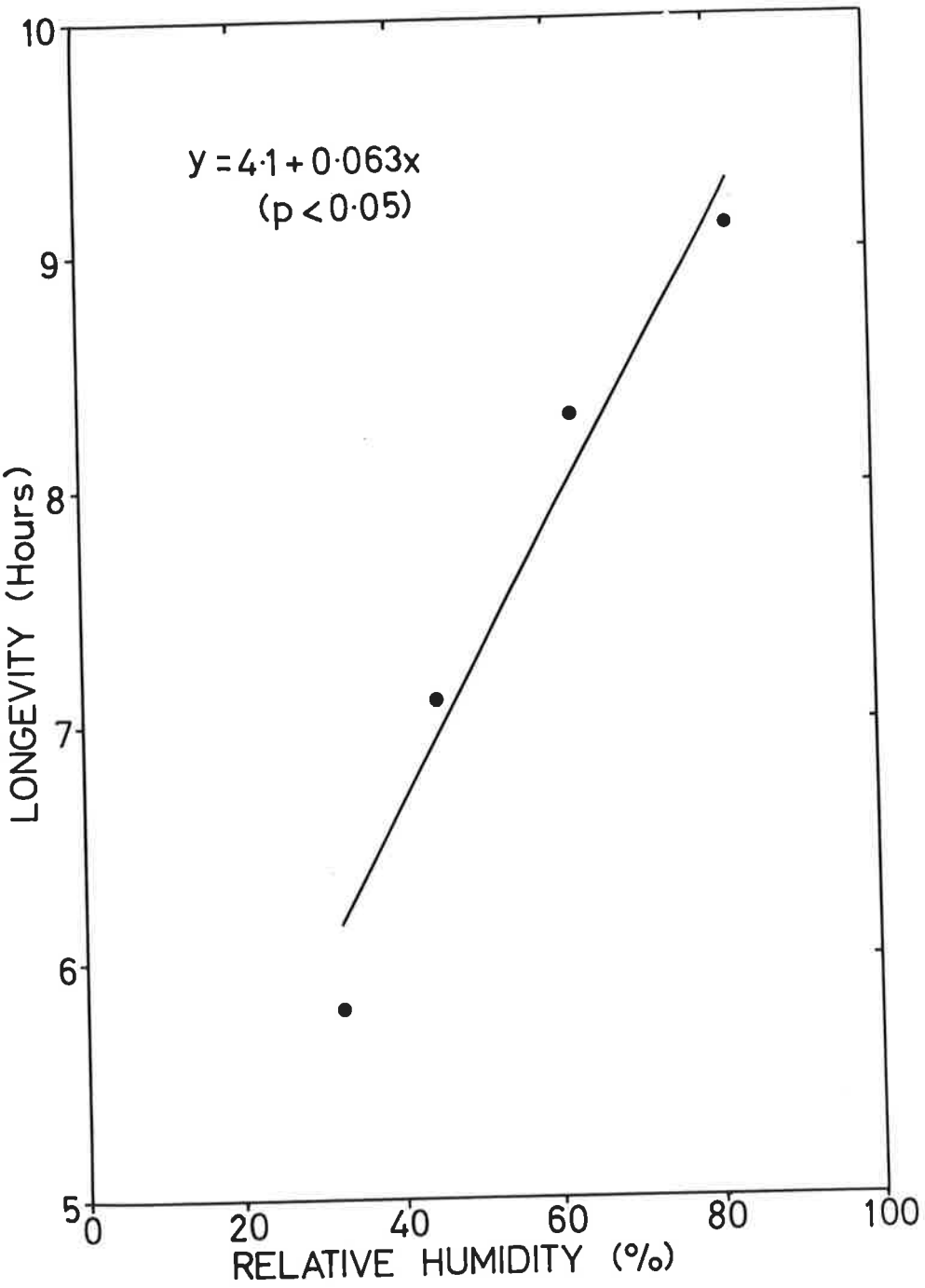

y = 24·1 - 0·54x
(p<0·01)
LONGEVITY (Hours)
TEMPERATURE
0
5
10
15
20
10
15
20
25
30
35
40

Table 3.7 Effect of humidity on male longevity of California red scale.

Humidity	n	Longevity (hours) Mean ± S.E.	Range
30-35%	20	5.8 ± 0.8	2-12
40-45%	20	7.1 ± 0.3	2-14
60-65%	20	8.3 ± 0.5	3-17
80-85%	20	9.1 ± 0.7	4-20

Fig. 3.4 Male longevity at various relative humidities.


y = 4·1 + 0·063x
(p < 0·05)
LONGEVITY (Hours)
RELATIVE HUMIDITY (%)
10
9
8
7
6
5
0
20
40
60
80
100

were dead within 4 h. Similarly, it was found in the field that most of the males die shortly after emergence during dry weather.

3.1.9 Effect of light intensity on male longevity

Tashiro and Beavers (1968) reported that males of red scale held in darkness. Some of these males lived 28 h at 25°C and 42 h at 10°C.

To investigate the influence of light intensity on longevity, emerging males were kept one to a petri dish and exposed to different light intensities ranging from 25 to 1000 lm./ft.^{2} at 25°C in groups of 20. Observations were made at hourly intervals until all were dead. The results are summarized in Table 3.8 and a linear regression of mean longevity on light intensity is illustrated in Fig. 3.5.

The results showed that the longevity of males was longest at the lowest light intensity and decreased with increase in light intensity, possibly due to the reduced activity of males at lower light intensities.

3.1.10 Effect of mating on male longevity

Quayle (1911a) and Jones (1963) reported that the unfertilized females of red scale lived much longer than fertilized females.

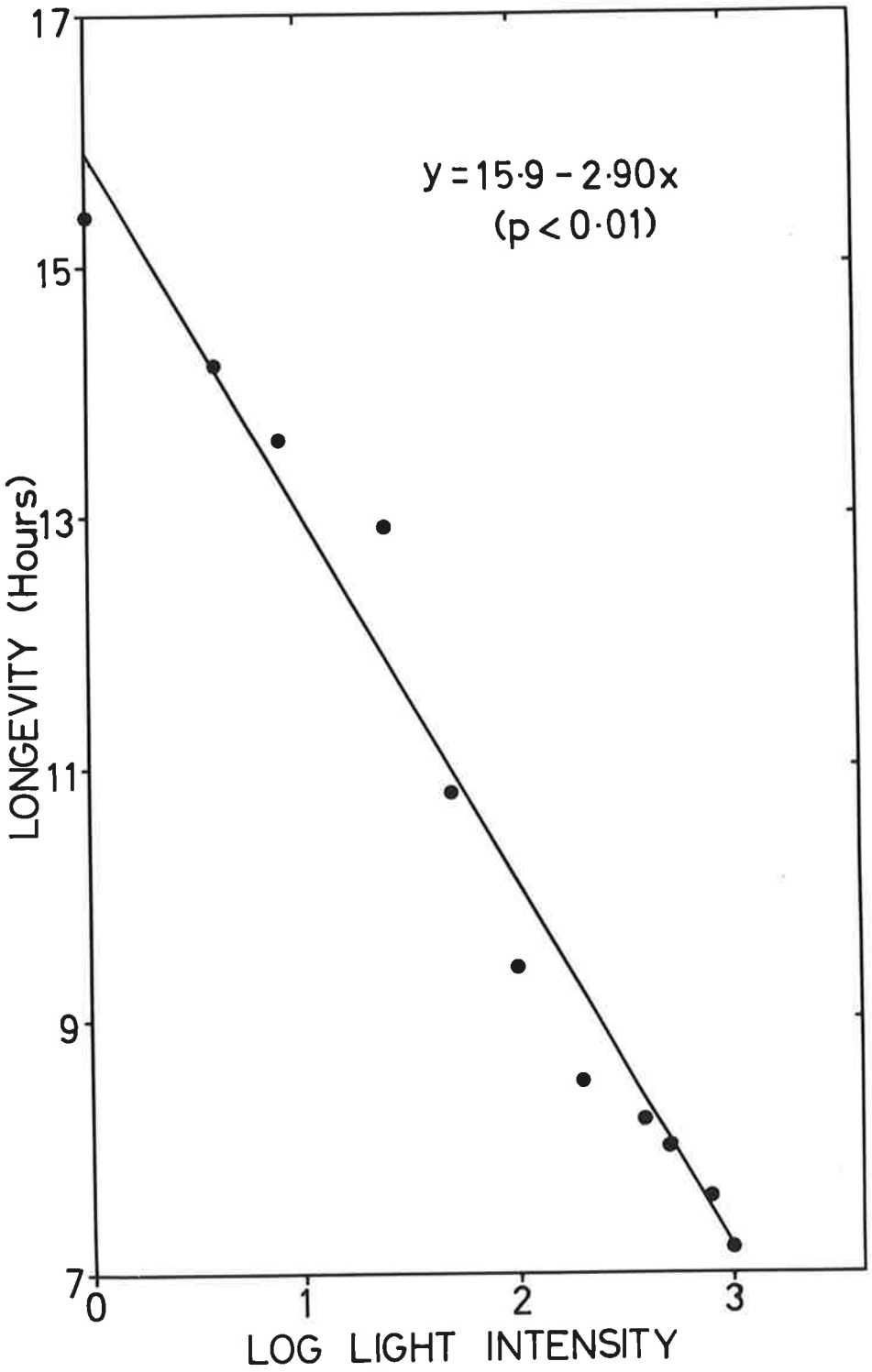
An experiment was performed to determine whether there was any difference in mean survival time between mated and unmated males of red scale. Three conditions were tested: condition 1 - male unmated (control); condition 2 - male mated to one female; and condition 3 - male mated to two females.

Emerging males were held once to a cage containing a leaf disc with one female, two females and without female in groups of 20. All the males

Table 3.8 Effect of light intensity on male longevity of California red scale.

Light intensity (lm./ft.^{2})	Log_{10}	n	Longevity (hours) Mean ± S.E.	Range
1	0	20	15.4 ± 0.6	7-31
4	0.6	20	13.9 ± 0.3	8-27
8	0.9	20	13.3 ± 0.8	7-26
25	1.4	20	12.6 ± 1.1	6-24
50	1.7	20	10.8 ± 1.0	4-21
100	2.0	20	9.4 ± 0.8	3-19
200	2.3	20	8.7 ± 0.3	4-16
400	2.6	20	8.3 ± 0.7	3-16
600	2.7	20	8.0 ± 0.6	3-13
800	2.9	20	7.6 ± 1.1	3-10
1000	3.0	20	7.2 ± 0.5	2-11

Fig. 3.5 Male longevity at various light intensities.


y = 15·9 - 2·90x
(p < 0·01)
17
15
13
11
9
7
0
1
2
3
LONGEVITY (Hours)
LOG LIGHT INTENSITY

moved around rapidly within minutes after their introduction to the leaf discs. Observations were made at hourly intervals until all the males were dead. The frequency of copulation of each male was determined by whether or not the females later produced crawlers.

Summaries of the longevity of males held under these conditions are given in Table 3.9. The results showed that longevity was significantly longer (at 0.05 level of probability) for unmated males than for those that had mated.

The longevity of males in the presence or absence of females was tested. The emerging males were placed in Munger cells attached to a lemon, some cells were placed in contact with sexually mature virgin females, such that the males could sense the presence of the female but not mate with them. Some cells were kept on lemon without any other scale, male or female. All cells were covered with nylon screen, and were held at room temperature (25°C) and at an illumination of 200 lm./ft.^{2}. Observations were made hourly until all males were dead (Table 3.10). No significant difference was noted between the longevity of the male in the presence or absence of females. Mortality commenced 3 h after male emergence, about half the males were dead within 7-8 h.

3.2 The mating behaviour of males

3.2.1 Introduction

Observations on mating behaviour in some species of *Aphytis* have been reported by Rao and Debach (1969a), wherein sexual behaviour leading to successful copulation was determined to be of a more or less set pattern. Mating occurs freely in the laboratory at any time of day or night, unlike some species of Lepidoptera where light, size of rearing unit, or time of

Table 3.9 Effect of numbers of mates on male longevity of California red scale.

	n	Longevity (hours) Mean ± S.E.	Range
Unmated	20	8.7 ± 0.8	3-15
Mated with one female	19	6.2 ± 0.6	2-11
Mated with two females	11	5.1 ± 0.7	2-8

Kruskal Wallis H Test ($P<0.05$).

Table 3.10 Effect of the presence of mature virgin females on male longevity of California red scale.

Virgin females	n	Longevity (hours) Mean ± S.E.	Range
Absence	20	8.6 ± 0.7	3-15
Prescence	20	8.2 ± 0.6	3-13

Mann Whitney U Test (P>0.05).

day or night have determined mating success (Shorey and Gaston, 1964).

Environmental factors such as temperatures, relative humidity and light may be expected to control the mating activities of red scales in at least two-ways. First, subthreshold levels of such factors may prevent mating; Callahan(1962) found that copulation occurred among adults of Heliothis zea (Boddie) only under low light intensities and high relative humidities, and when the air temperature was below 30°C. Second, diurnally fluctuating environmental factors may possibly control the timing of copulatory activity (Saario et al., 1970; Sanders et al., 1972; Carde' et al., 1975; Castrovillo and Carde', 1979).

The research reported herein was conducted to determine the effect of selected environmental factors upon the occurrence and timing of mating of the males.

3.2.2 Materials and Methods

Five crawlers were transferred to each disc the same morning and kept at 25°C and 70±5% ambient RH. The light regime was 12L:12D and the photophase light intensity was 400 lm./ft.^{2}.

After the sexes become distinguishable, one female was selected from each disc and all others removed. Males obtained from the collection chamber were immediately placed one to a cage containing a leaf disc with a mature virgin female and held under the conditions of the treatment under tests.

For the purpose of experiments it was desirable to obtain large number of newly emerged males early in the afternoon. However, as indicated previously (section 3.1.3) under natural conditions most males

emerged between 17.00 and 18.30 h. For this reason the following procedure was adopted. Lemons bearing adult males were kept for 24 h in complete darkness (in several fiberboard cartons) which had the effect of inhibiting emergence (Tashiro and Beavers, 1968). When such males were subsequently placed under a bank of fluorescent light, the strong illumination evidently stimulated the sudden release of the males, and it was possible to obtain the numbers required for experiments at the most suitable time.

3.2.3 Time of mating of the males

Tashiro (1968) found that almost all the males emerged and were in flight in late afternoon, but because the longevity of males is very short (approximately 6 h), the copulatory activity must occur shortly after emergence. Quayle (1911) stated that copulation may occur within ½-1 h after emergence. An experiment was conducted to observe the time of mating of the males in the laboratory and outdoors.

3.2.3.1 Laboratory observations

Twenty males were each introduced to a separate cage containing a female, and every 30 min moved to a new cage with another female. This continued until all the males were dead. The time of copulation of each male was determined by the females that produced crawlers.

Typically, the male approached the female in an erratic manner, repeatedly passing or returning away and then returning immediately; activity was continuous and unhurried. Copulation was attempted by downward positioning of the aedeagus and a circling movement as though the male was orienting (Tashiro and Chambers, 1967).

Copulation commenced at 15.30 h, 30 min after male emergence, and reached its peak within one hour and ceased within four hours. The times

of copulation are plotted in Figure 3.6A.

3.2.3.2 Outdoor experiment

The experiment was repeated with the cages outdoors. A photometer and Wallac thermometer were used to measure the levels of illumination and temperature (Table 3.11).

Copulation commenced one hour after male emergence, reached its peak within two hours, and ceased within four and half hours at 19.30 h. The times of copulation for males are plotted in Figure 3.6B.

The mean time of copulation was delayed by about half an hour compared with laboratory conditions. This may have been due to the fluctuating environmental factors.

3.2.4 Effect of temperature on mating

Sower at al. (1970) have shown that the mating of cabbage looper in the laboratory is advanced by lowering the temperature; this has also been noted by Sanders and Lucuik (1972) in the Spruce budworm Choristoneura funiferana (Clem); Cardé and Roelofs (1973) in the arctiid moth, Holomelina immaculata (Reakirt); Cardé et al. (1975) in the Redbanded leaf-roller, Argyrotaenia velutinana (Walker). The daily change in temperature, together with falling light intensity may have modified male activity and mating behaviour.

An experiment was carried out to determine the effect of temperature on male mating. Emerging males were held one to a cage containing a leaf disc with a virgin female at the experimental temperatures of 10, 15, 20, 25, 30 and 35°C in 12L:12D. One hundred males were observed at each temperature. The relation between temperature and percentage of mating

Fig. 3.6 Timing of copulation of California red scale.

Males that emerged at 15.00 h.

A: Adult males held in cages in the laboratory at a constant temperature 25°C and light 400 lm./ft.^{2} (humidity : 70 ± 5% RH).

B: Adult males held in cages outdoors.

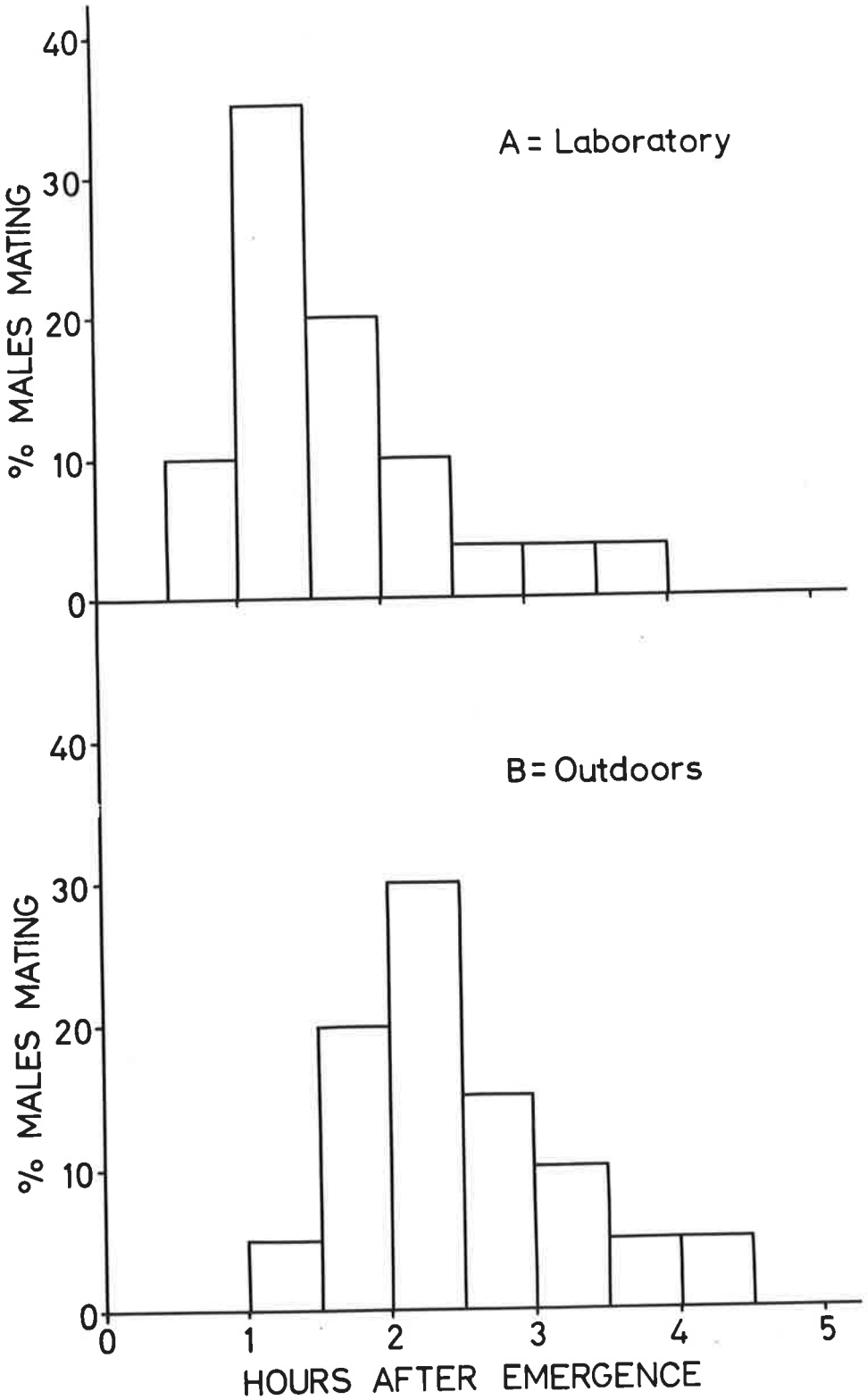

A = Laboratory
B = Outdoors
% MALES MATING
40
30
20
10
0
0
1
2
3
4
5
HOURS AFTER EMERGENCE

Table 3.11 Temperatures and levels of illumination at the times of day at which male copulatory activity was tested, as indicated in Fig. 3.5B.

Time[a]	Levels of illumination (lm./ft.^{2})	Mean temperature (°C)
15.00	825	31
15.30	785	31
16.00	670	29
16.30	590	28
17.00	535	26
17.30	450	24
18.00	370	23
18.30	320	23
19.00	175	21
19.30	60	19
20.00	15	18
20.30	<1	17
Night	>0.1	14

a) Pacific daylight time.

is summarized in Figure 3.7.

The results obtained from this experiment indicated that an increase of temperature brought about an increase in the proportion of males that copulated successfully. A temperature of 40°C was also tested, but very few males mated, and most of them were dead within a few hours.

3.2.5 Effect of light intensity on mating

Shorey and Gaston (1964, 1965a) demonstrated that light affects the occurrence and the timing of response of males of cabbage looper to the female sex pheromone. Male pheromone responsiveness was greatly inhibited by light intensities higher than that of bright moonlight.

The influence of various light intensities on the occurrence of mating was studied in a laboratory maintained at 25°C. Light intensities of 400, 25, 8, 4, 2, 1 and 0.5 lm./ft.^{2} were obtained by decreasing the vertical distance of the leaf discs from the center of a bank of fluorescent tubes.

Emerging males were held one to a cage containing a leaf disc with a virgin female at the experimental light intensities. One hundred males were observed at each intensity. The relation between light intensity and the percentage of males that mated is summarized in Fig. 3.8.

The results showed that the copulation of males was highest at 400 lm./ft.^{2} and rapidly decreased with decrease in light intensity. At the lowest light intensity of 0.5 lm./ft.^{2}, only 8% of males mated.

3.2.6 The frequency of copulation

Bodenheimer (1951) indicated that males probably mated only once,

Fig. 3.7 Effect of various constant temperatures on the percentage mating of males.

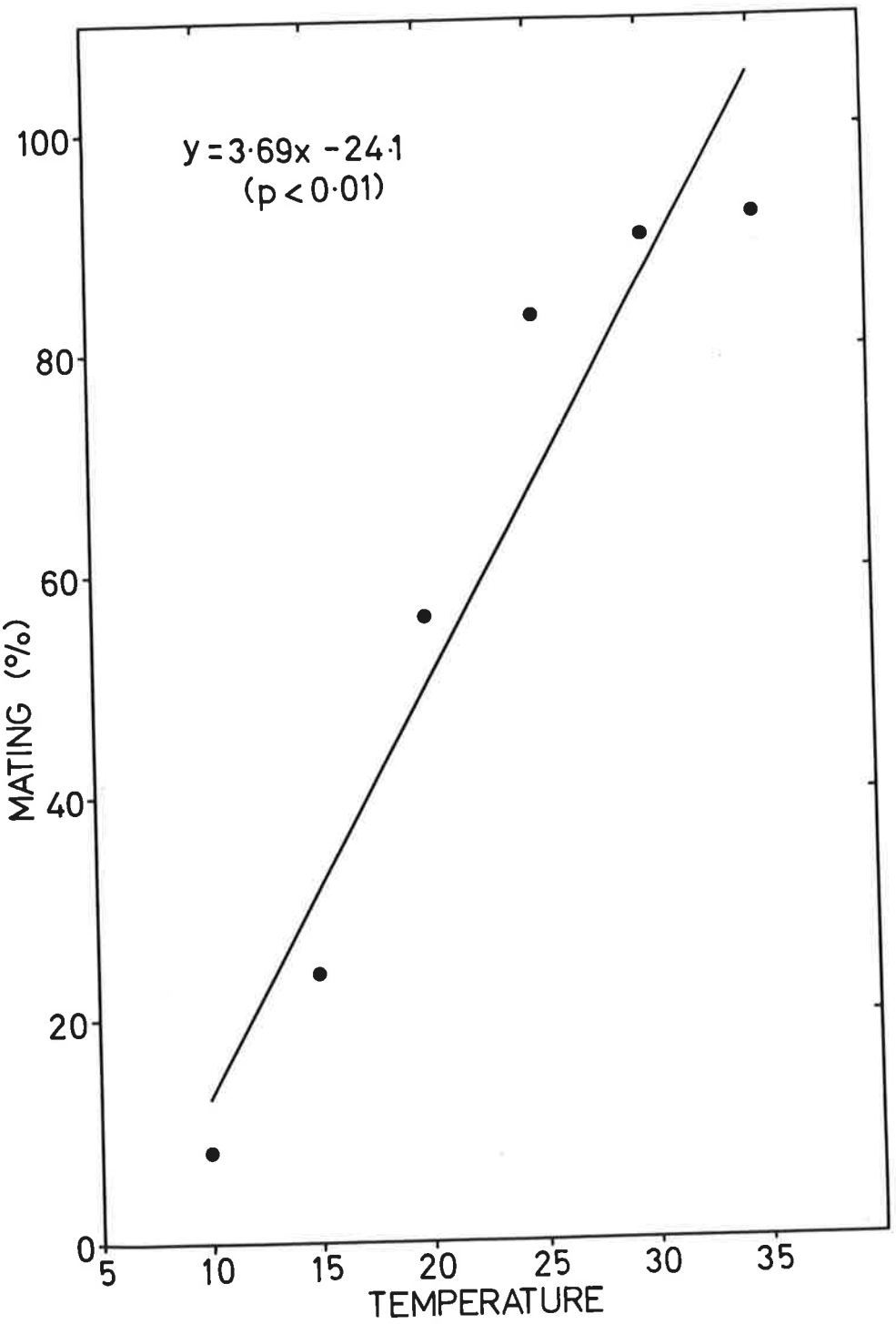
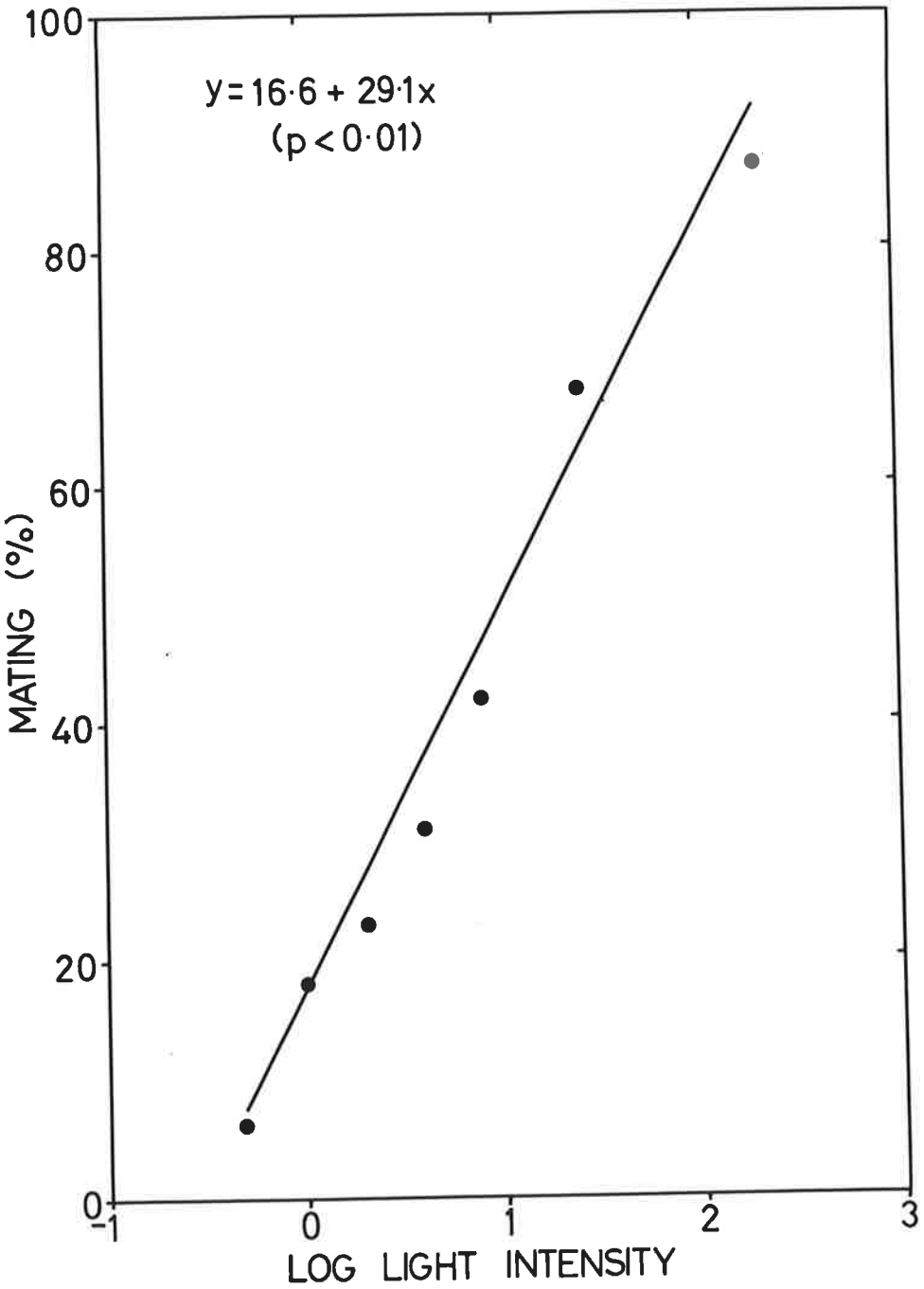

y = 3·69x - 24·1
(p < 0·01)
100
80
60
40
20
0
MATING (%)
5
10
15
20
25
30
35
TEMPERATURE

Fig. 3.8 Effect of various constant intensities of light on the percentage of males mating.


y = 16·6 + 29·1x
(p < 0·01)
100
80
60
40
20
0
MATING (%)
−1
0
1
2
3
LOG LIGHT INTENSITY

but exploratory experiments conducted by A.W. Cressman of his laboratory (unpublished data) indicated that each male was capable of mating with about 4 females, and Tashiro et al. (1968) reported that a male had inseminated as many as 11 females.

An experiment was conducted to estimate the mating frequency of males. Forty emerging males were held one to a cage containing a leaf disc with 20 virgin females, in the laboratory at 25°C, 70±5% RH and 400 lm./ft.^{2} light intensity.

All the males moved around rapidly within minutes after their introduction to the leaf discs. The frequency of copulation of each male was determined by production of crawlers by the females. The distribution of mating frequencies for 40 males are summarized in Table 3.12.

The mean copulation frequency for all 40 males was 2.1, and the maximum copulation frequency was five times. The first mating generally occurred at 30 min after male emergence, and mating creased within four hours.

3.3 Conclusion and Discussion

Results of emergence test conducted in the field indicated that most of the males emerged and flew in the late afternoon. Emergence was influenced by the levels of illumination and temperature, with a peak of emergence occurring at about 510-325 lm./ft.^{2} and 25-26°C.

The test in the laboratory shown that with higher temperatures (in the range 15 to 35°C) the peak of emergence occurred closed to the midday. When the temperature remained at 10°C or less, there was

Table 3.12 Distribution of mating frequencies for 40 red scale males separately caged throughout their lives with 10 of mature virgin females.

Number of matings	Frequency of males	
	Number	Percentage
0	1	2.5
1	14	35
2	12	30
3	8	20
4	3	7.5
5	2	5.0

no response to light, probably because the temperature was below the activity level of the male for emergence. Similarly with an increase of temperature in the absence of light emergence did not occur.

It was shown that the daily rhythm of emergence of male red scale is entrained by an interaction between the light and temperature cycle. The light is apparently the critical cue for the release of emergence, with darkness or extreme high and low temperature inhibiting it.

McLaughlin and Ashley (1977) also found that the daily rhythm of eclosion of male white peach scale, Pseudaulacaspis pentagona is entrained by the interaction of the prevailing light and temperature cycles. The eclosion rhythm is modified daily by the prevailing temperature cycle, apparently to ensure maximum survival of, and mating by the short-lived males.

Male red scale are short-lived (Tashiro and Beavers, 1968) and must quickly and efficiently locate and mate with mature females. Thus, in the field the daily synchronization of their emergence with declining temperature, usually during the afternoon or early evening, assures that they will avoid the extreme heat of the day but emerge while temperatures are still high enough to sustain activity. Optimum flight temperature for male red scale is c. 27°C (Rice and Moreno, 1970), and the field activity of the yellow scale, Aonidiella citrina (Coquillett) (Moreno et al., 1974) suggest that their eclosion may be regulated like that of red scale by photoperiod and temperature.

The longevity of male was longest at the lowest light intensity and temperature. Tashiro and Beavers (1968) also found that males held in darkness lived 28 h at 25°C and 42 h at 10°C. Possibly due

to the reduced activity of males at lowest intensity and temperature. Survival of unmated males was significantly longer than the mated males (Table 3.9). It seems that the rate of activity of mated males slows down, while unmated ones continue to search for females.

Regulation of mating activity of red scale is affected by both light and temperature. Copulatory activity of males was found to increase with light intensity and temperature. This correlation does not necessarily indicate that the inhibition of mating at lower light intensity and temperature are caused entirely by reduced male responsiveness; it could be attributable to a reduction in the tendency of females either to release pheromone or to be receptive to the males.

Carde' et al. (1975) indicated that the interactions of temperature and light cycles in affecting the initiation and duration of female calling behaviour and male responsiveness are complex. In the tortricid moth, Argyrotaenia velutinana a decrease in temperature occurring within a specific daily gate can induce both female calling and male responsiveness. Cool temperature are undersirable because they increase the demand for metabolic energy necessary to sustain mating flight (Henegan and Heath, 1970). This consideration is most crucial in small insects that possess a high surface area to volume ratio.

In the field, temperature usually fall as the light intensity falls and few males copulated at temperatures below about 15°C. Even in summer, 15°C is not unusual at sundown in South Australia. So virtually all seasonal activity would be inhibited by both low temperature and darkness.

CHAPTER 4

DEVELOPMENT OF METHODS OF ASSAYING SEX-PHEROMONE AND DETERMINATION OF THE BEHAVIOUR OF CALIFORNIA RED SCALE MALES UNDER THE INFLUENCE OF FEMALE PHEROMONE

4.1 Introduction

Sex attractant traps baited with either living virgin females, sex pheromone extracted from virgin females, or synthesized sex pheromone, have been used in many areas for insect survey and detection (Collins and Potts, 1932; Proverbs, 1965; Dean and Roelofs, 1970; Mitchell and Hardee, 1974; Sanders, 1978; Carde', 1979; Gardner et al., 1983; and Moreno, 1983). They have also been used to study population structure, the effects of sterilization and marking on laboratory-reared insect, effects of laboratory rearing, and control (Gaston et al., 1967; Wong and Cleveland, 1970; Schwable, 1979; Elkinton and Carde', 1980). So far, information on sex pheromones has provided us with a useful device in the control programs for a relatively few harmful, such as pink bollworm, Pectinophora gossypiella (Saunders); gypsy moth, Lymantria dispar (L.); and cabbage lopper moth, Trichoplusia ni (Hubner).

The discovery of a female sex pheromone in the California red scale, and the extraction and bioassay of several preparations of the pheromone have been reported (Tashiro and Chambers, 1967; and Tashiro et al., 1969). However, a more suitable method of bioassaying the pheromone was needed, particularly to expose virgin females and the pheromone to larger numbers of males to increase the validity of evaluations. Since such evaluation of extractants, including pheromones, is usually done by employing an olfactometer, an attempt was made to develop a suitable apparatus and technique for bioassay.

4.2 MATERIALS, METHODS AND RESULTS

4.2.1 Bioassay of the female sex pheromone of red scale

4.2.1.1 T-tube olfactometer for bioassay

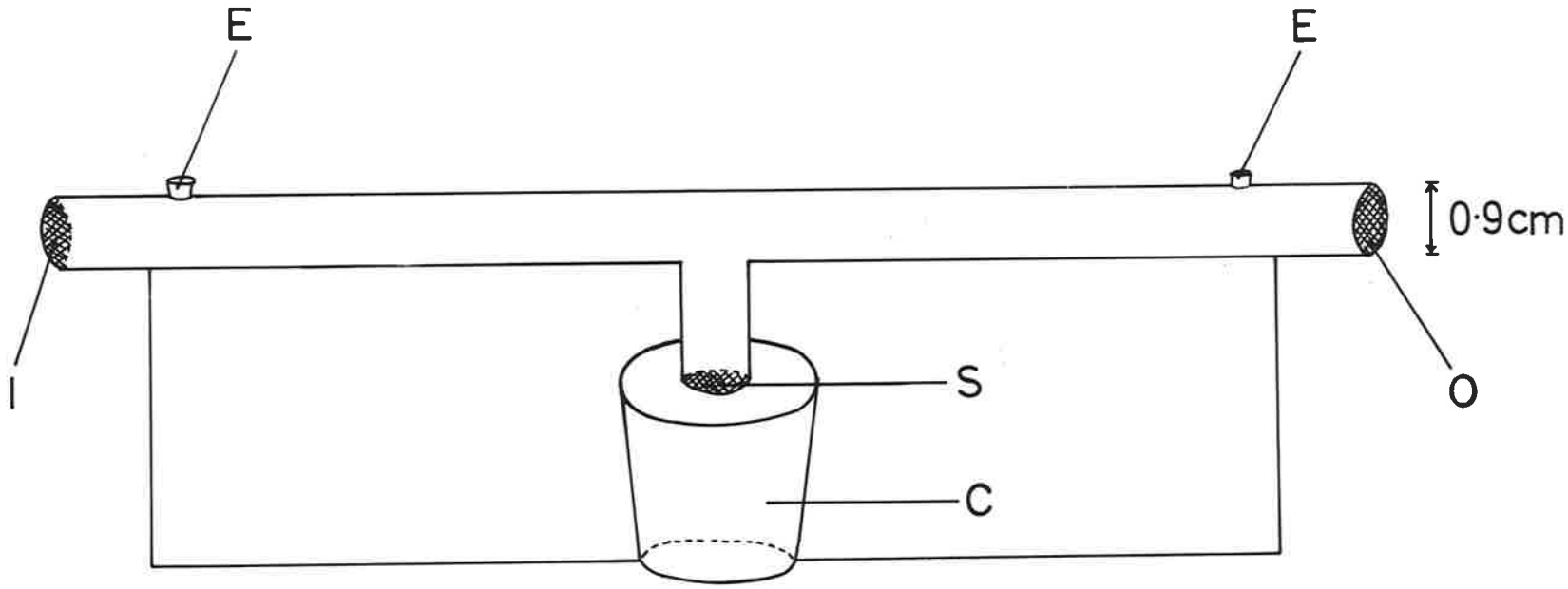
A "T-tube" olfactometer was designed and used for testing of the pheromone. Fig. 4.1 shows a schematic diagram of the apparatus. It was made from a glass tube 40 cm long, 0.9 cm in diameter with a central tube 6 cm long fixed at right angles. The ends of the 40 cm tube were covered with fine screen that permited air to move through the tube. Two removable cork plugs (0.8 cm-diam) were inserted into holes in the arms of the 40 cm tube about 5 cm from the end, through which males could be introduced into the tube. The source chamber was a plastic vial 8 X 9½ cm, whose lid was provided with a small hole (0.9 cm-diam), plugged with fine gauze. The testing was conducted in a blackened wooden box, 35 X 22 X 17 cm, at 25°C and 75±0.5% RH with illumination of 120 lm./ft.^{2} provided by fluorescent and incandescent lamps. During the operation, a constant, slow stream of air (1 litre/min) was drawn through one long arm of the tube.

Virgin females were held in isolation until they were sexually mature, then during the afternoon when males normally emerge and mate, 50 virgin females were removed from lemons and crushed in 1 ml of each of 9 common solvents. The breis were held in small seald containers at 2°C until needed.

The testing procedure was as follows: (1) emerging males were introduced, one at a time into the hole of the tube. (2) One replicate of solution containing the desired concentrations of pheromone was pre-applied to 4.25 cm pieces of Whatman No.1® filter paper and sealed

Fig. 4.1 Schematic diagram of a "T-tube" olfactometer (side view).

I: Air inlet screen
O: Air outlet screen
E: Plug for loading males
C: Source chamber
S: Screen for source through the tube


E
E
0·9cm
I
S
O
C

in plastic vials as soon as the odour of the solvent was no longer noticeable. (3) At the end of 15 min, the pheromone source was sealed off. (4) Then the males were counted in the arm or on the screen of the source chamber and removed with a brush. (5) Males were also introduced from the arm upwind to observe the behaviour of males in the absence of pheromone. (6) All tests were conducted in the afternoon between 14.00 and 18.00 h during the normal mating period.

The presence of the pheromone was demonstrated by the males being attracted to the screen of the source chamber and attempting to mate. High attraction was obtained with extracts in diethyl ether, acetone, chloroform, methylene chloride, n-hexane and petroleum ether. Eight of nine preparations elicited a copulatory response, an indication that many solvents may be capable of extracting the pheromone with varying efficiency. Preparations of acetone, diethyl ether and methylene chloride were the most efficient. The responses are summarized in Table 4.1. No males were attracted in the absence of pheromone.

4.2.1.2 Design of the "Sticky slide trap" for bioassay

A sticky slide trap was constructed to compare the attractiveness to males of extracts of pheromone with virgin females. The males were allowed to fly freely in a chamber in a glasshouse (5 X 4 X 3.5 m) maintained at 25-26°C.

The trap (Fig. 4.2) was a 12 cm cube with solid wood top and bottom. Each of the slides was provided with a frame into which could be slotted five 7.5 X 2.5 cm glass microscope slides in louvre fashion. The slides were held at an angle of 45° to allow maximum deposition of wind-borne particles (Gregory and Stedman, 1953), and only the upper surface of

Table 4.1 Attractiveness of extracts of sex pheromone of red scale to males on the T-tube olfactometer.

Solvent	Average number of males[a] responding		
	Attraction[b]	Response[c]	Non-response[d]
Methylene chloride	16	4	0
Diethyl ether	15	5	0
Acetone	15	4	1
Petroleum ether	14	4	2
Chloroform	12	6	2
Hexane	10	7	3
Benzene	7	9	4
Ethyl alcohol	6	7	7
Water	2	7	11

a) Based on 20 males tested per extract

b) Males attracted to the screen of source chamber and attempting to mate

c) Males which repeatedly returned to the site of source without attempting to mate

d) Males moving upwind but not reaching the pheromome source.

Fig. 4.2 Sticky slide trap

the slides were coated with tanglefoot. The source of pheromone, either a lemon with virgin females (Fig. 4.3), or a vial containing the diethyl ether and methylene chloride extracts of pheromone was placed in the centre of the cube.

For exploratory studies, groups of 5, 20 and 100 virgin females reared on lemons were compared with two extracts of the females sex pheromone. Traps were hung 2 m high and 4 m away from the release point of the males, and 15 cm apart from each other. The treatment was tested in the afternoon between 14.00 and 18.00 h, four replications per attractant in each test, and the test was repeated on two consecutive afternoons. One hundred emerging males were released in each treatment, and all males attracted in the traps were counted. The attraction of males is summarized in Table 4.2.

Data from the test (Table 4.2) show that 100 virgin females trap was significantly attracting more males than others. Traps containing the diethyl ether and methylene chloride extracts of pheromone were about as attractive as 20 virgin females. The extremely low numbers of males attracted to the controls gave an indication of the response to the pheromone.

4.2.2 Effect of dispenser dose on attractiveness

Tests were conducted to determine whether a different concentration of preparation of pheromone would give similar results.

Sand was used as the substrate for the pheromone in all tests. The mixture of sand and pheromone was prepared by pipetting a known volume of diethyl ether extract of pheromone onto 20 g of sand and

Fig. 4.3 A lemon with virgin females was placed in the centre of the cube.

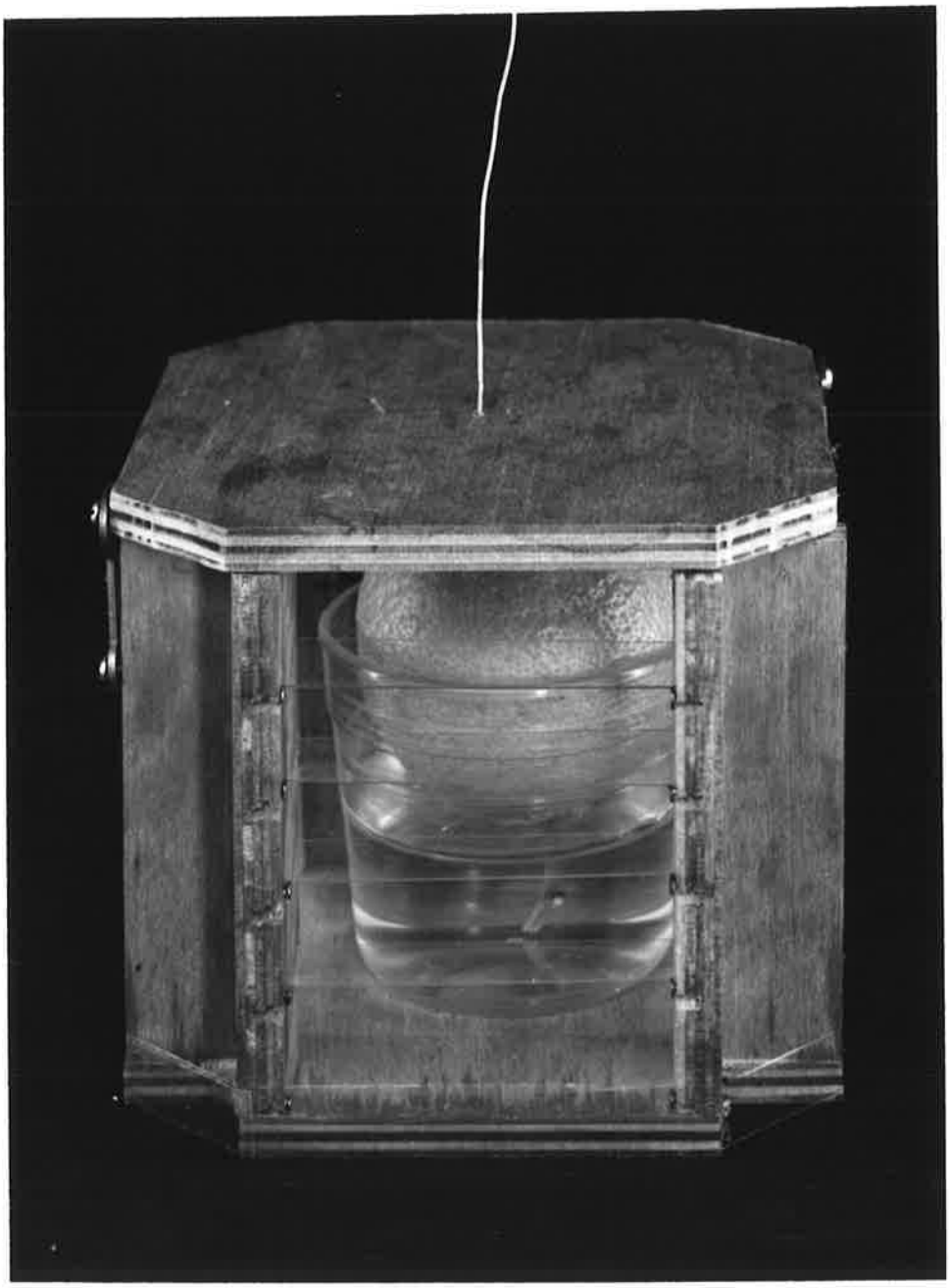


Table 4.2 Attractiveness of extracts of sex pheromone compared with that of live virgin females of red scale on the sticky slide trap. Four replications per attractant in each test.

Attractant	% of total number of males[a] attracted[b]	
	Test 1	Test 2
Methylene chloride extract	15c	17b
Diethyl ether extract	14c	15b
5 females	7d	9c
20 females	18b	16b
100 females	46a	43a
Control (lemon alone)	<1d	<1d

a) Total of 356 and 341 of males counted in test 1 and 2.

b) Means within columns followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Both extracts of pheromone 10 mg were pipetted onto paraffin drops on the surface of lemons.

stirring it to ensure uniform distribution. A plastic dish (1 cm high X 4.5 cm diam) were usually used to contain the treated sand in the trap.

All treatments were tested on single individuals in the afternoon, and each treatment was replicated 4 times. One hundred emerging males were released in each treatment. The numbers of males captured in traps baited with 1000, 100, 10, 1 or 0.1 mg of pheromone are summarized in Table 4.3.

Data from the test (Table 4.3) show that 1000 mg pheromone attracted more males than either 10, or 1, and 0.1 mg. Males responded much the same to the 1000 mg concentration and 100 mg concentration, which may indicate saturation of male responsiveness at the highest dosages. Shorey (1967) suggested that inhibition of response occurs at higher concentrations, and the field results of Wolf et al. (1967) also indicated the possibility of such inhibition.

4.2.3 Attractiveness of different number of females

An experiment was conducted to estimate the attractiveness of female traps when varying number of virgin females were used in a glasshouse.

Varying numbers of virgin females, all of the same age (4-5 weeks old) were placed in the trap. The treatments were tested on single individuals in the afternoon between 14.00 and 18.00 h. One hundred emerging males were released in each test, and the traps were placed at height of 2 m, and 4 m away from the released males. The number of males attracted in traps baited with 5, 10, 25, 50, 100, 200 and 400 of virgin females are summarized in Table 4.4.

The results shown in Table 4.4 indicate that in the laboratory

Table 4.3 Effect of five concentrations of pheromone on the catch of male red scale in a sticky slide trap.

Amount of pheromone (mg)	Mean number of males attracted/trap[a]
1000	62.25a
100	60.50a
10	39.75b
1	26.50c
0.1	15.00d

a) Means followed by the same letter are not significantly different at the 5% level of confidence based on Duncan's multiple range test.

Table 4.4 Numbers of males caught in traps baited with different number of virgin females.

No. females used	Side of trap*				Total
	Right	Left	Front	Back	
0	1	0	2	0	3f
5	1	3	10	0	14ef
10	3	4	16	1	24de
25	7	6	23	0	36cd
50	6	9	27	1	43c
100	10	7	44	3	62b
200	12	10	53	3	78a
400	15	11	50	5	81a

One hundred males were released in each treatment

* Means followed by the same letter are not signficantly different at the 5% level of confidence based on Duncan's multiple range test.

tests there was little difference between the attractiveness of 200 and 400 females. However, increasing the number of virgin females from 5 to 200 attracted more males. There was a significant difference between the attractiveness of either right or left slide compared with the front of the traps. An average of 65% of males was attracted to the side of the traps nearest the release point (front); more or less equal number of males were attracted to both right and left sides of the traps, but very few were attracted to the side furthest from the release point (back).

4.2.4 Effect of female age on attractiveness

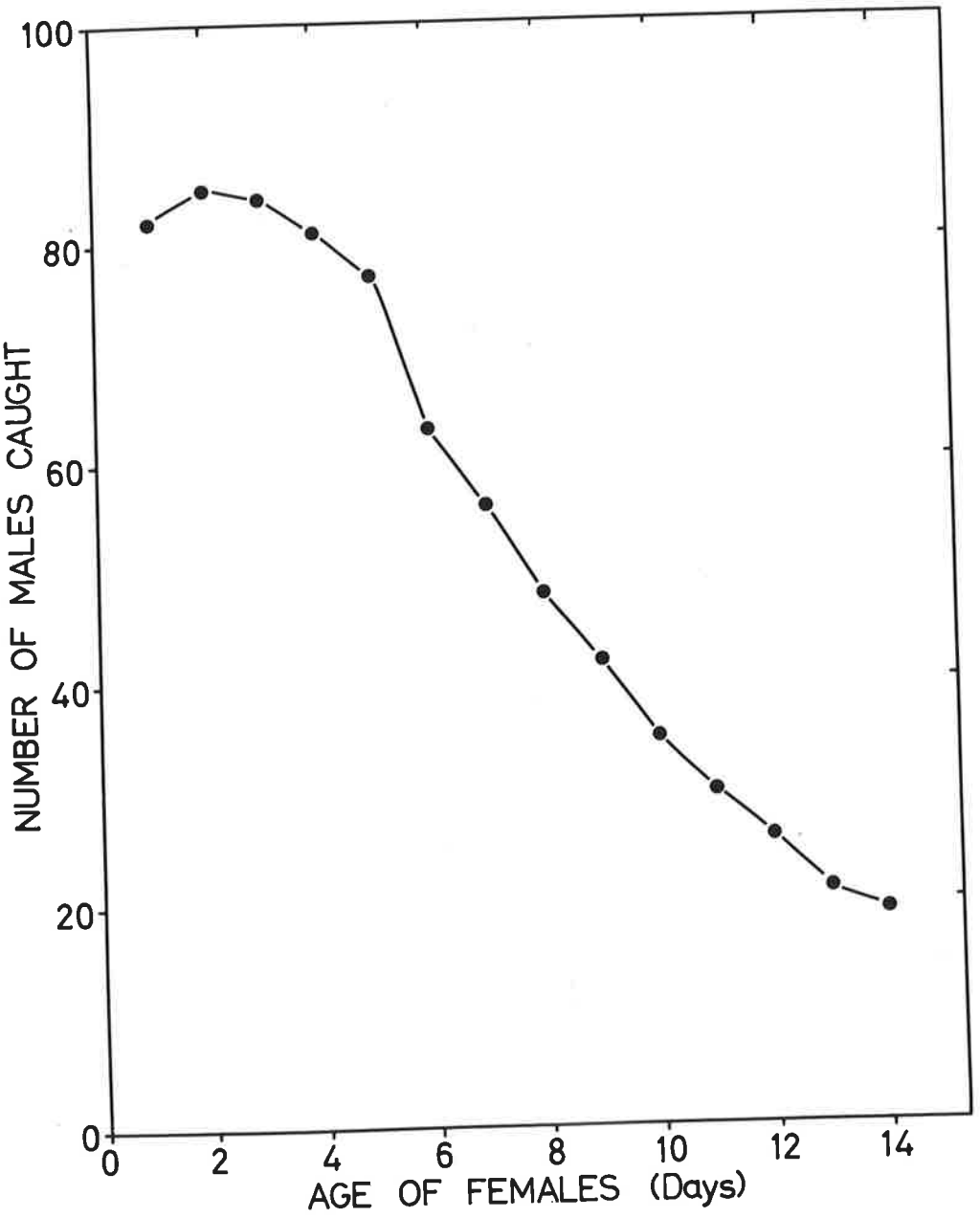
Charlton and Carde' (1982) stated that the female of gypsy moth, Lymantria dispar are most attractive on the second day after emergence. The eastern spruce budworm, Choristoneura fumiferana is most attractive 1-2 days old (Sanders and Lucuik, 1972).

An experiment was conducted to determine the effect of age on attractiveness. Two hundred virgin females of different ages were used in each treatment, and treatment were tested on single individuals in the afternoon between 14.00 and 18.00 h. One hundred emerging males were released in each test, the number of males attracted after 18.00 h was recorded. The results obtained, as shown in Fig. 4.4 indicate a marked decrease in attractiveness after the fifth day of female life.

4.2.5 Effect of male age on response to sex pheromone

The male response traps baited with virgin females depends upon many variable factors, amony the more important of which are age and physiological condition. For example, Stafford (1971) reported that the response of males of the omnivorous leaf-roller to sex attractant was affected by their age. It was noticed that one to four-day-old

Fig. 4.4 Numbers of the red scale males trapped by females of different ages.


100
80
60
40
20
0
NUMBER OF MALES CAUGHT
0
2
4
6
8
10
12
14
AGE OF FEMALES (Days)

males were most attracted to virgin females.

To determine the effect of age on the response, 100 males of different ages were released at each treatment. The treatments were tested on single individuals in the afternoon. Traps baited with 200 virgin females were used, and the numbers of males caught were recorded.

Almost all the males emerged and were in flight in late afternoon, but because the longevity of males is very short (average 6-7 h), the copulatory activity must occur shortly after emergence. The results shown in Fig. 4.5 indicate that the male response to sex attractant was affected by their age. It was shown that one and two-hour-old males were very attracted to virgin females. whereas the older males were not so excited by virgin females' sex lure. Very few 8-hour-old males were caught.

4.2.6 Effect of time of day on male response to virgin females

If peaks or lulls occurred in activity of either males or females during a given day the reliability of tests conducted throughout the day would be affected. A test was therefore made to determine whether time of day had any effect on male response.

A trap baited with 200 virgin females was used as a pheromone source, and placed at 2 m high above the floor. A cage of pre-emergence adult males reared on lemon was placed at 4 m away from the pheromone source. The attracted males were counted at hourly intervals during a 24 h period.

The results, as shown in Fig. 4.6, indicate that the males were attracted between midday and 20.00 h with a sharp peak between 16.00 and 18.00 h, trapping 49 males. About 56% of the males caught were

Fig. 4.5 Attractiveness of virgin females of the red scale to different ages of males.

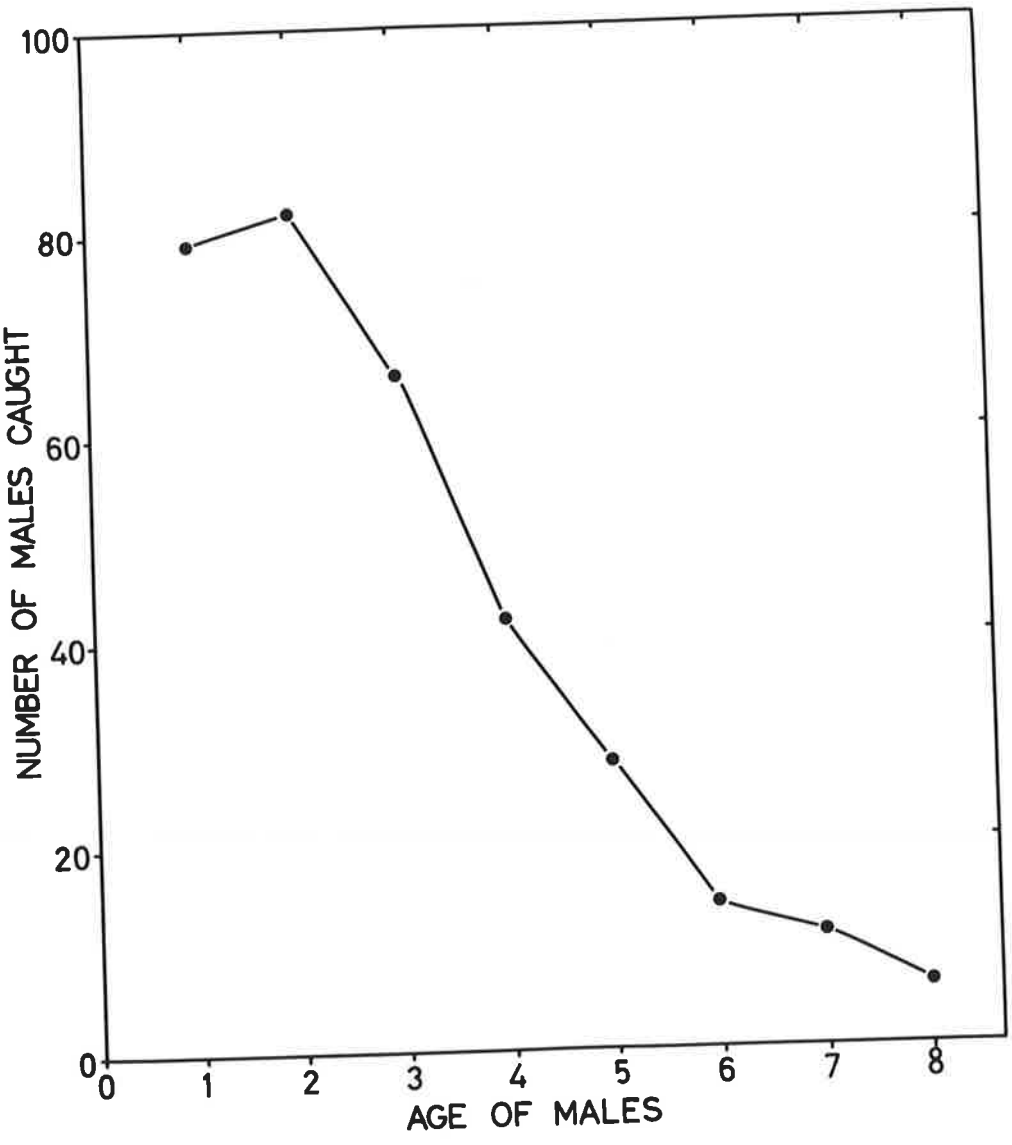
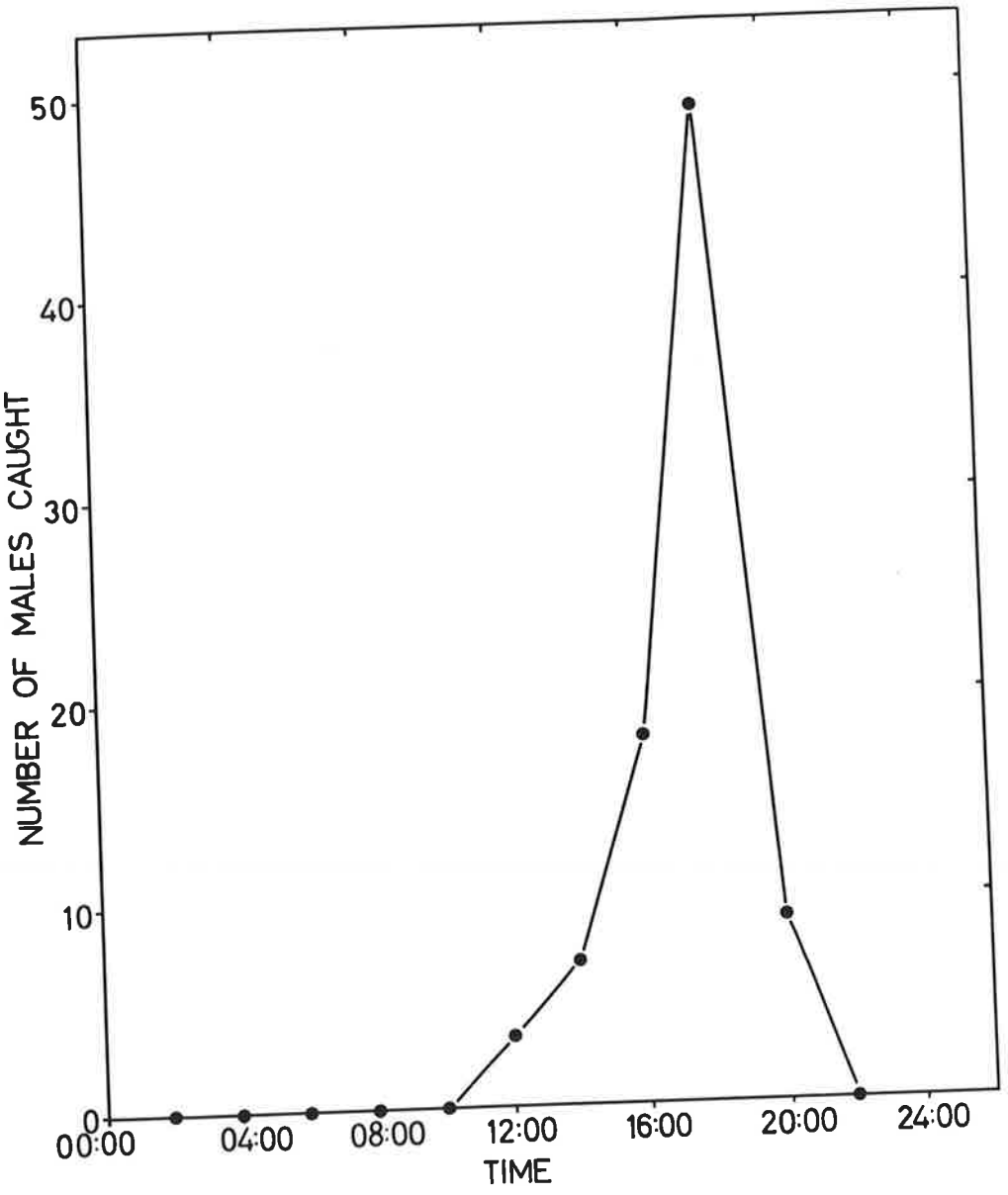

100
80
60
40
20
0
NUMBER OF MALES CAUGHT
0
1
2
3
4
5
6
7
8
AGE OF MALES

Fig. 4.6 Numbers of males caught in traps baited with virgin females within a 24 h period.


NUMBER OF MALES CAUGHT
50
40
30
20
10
0
00:00
04:00
08:00
12:00
16:00
20:00
24:00
TIME

attracted between 16.00 and 18.00 h, and no males were caught in the morning and at night. Sunset during the experimental day was at 20.30 h ± 5 min.

Fig. 4.7 shows changes in male attraction within the period 16.00 to 19.00 h. It indicates that more males were attracted between 16.30 and 18.30 h, with a peak at 16.45 h, trapping 8 males in 15 min. Readings taken every 15 min interval showed that male trapping increased rapidly between 16.45 and 17.45 h, then it declined slowly till 18.30 h. Only a few males were caught between 18.30 and 19.30 h. Sunset during the experimental days was at 20.40 h ± 5 min.

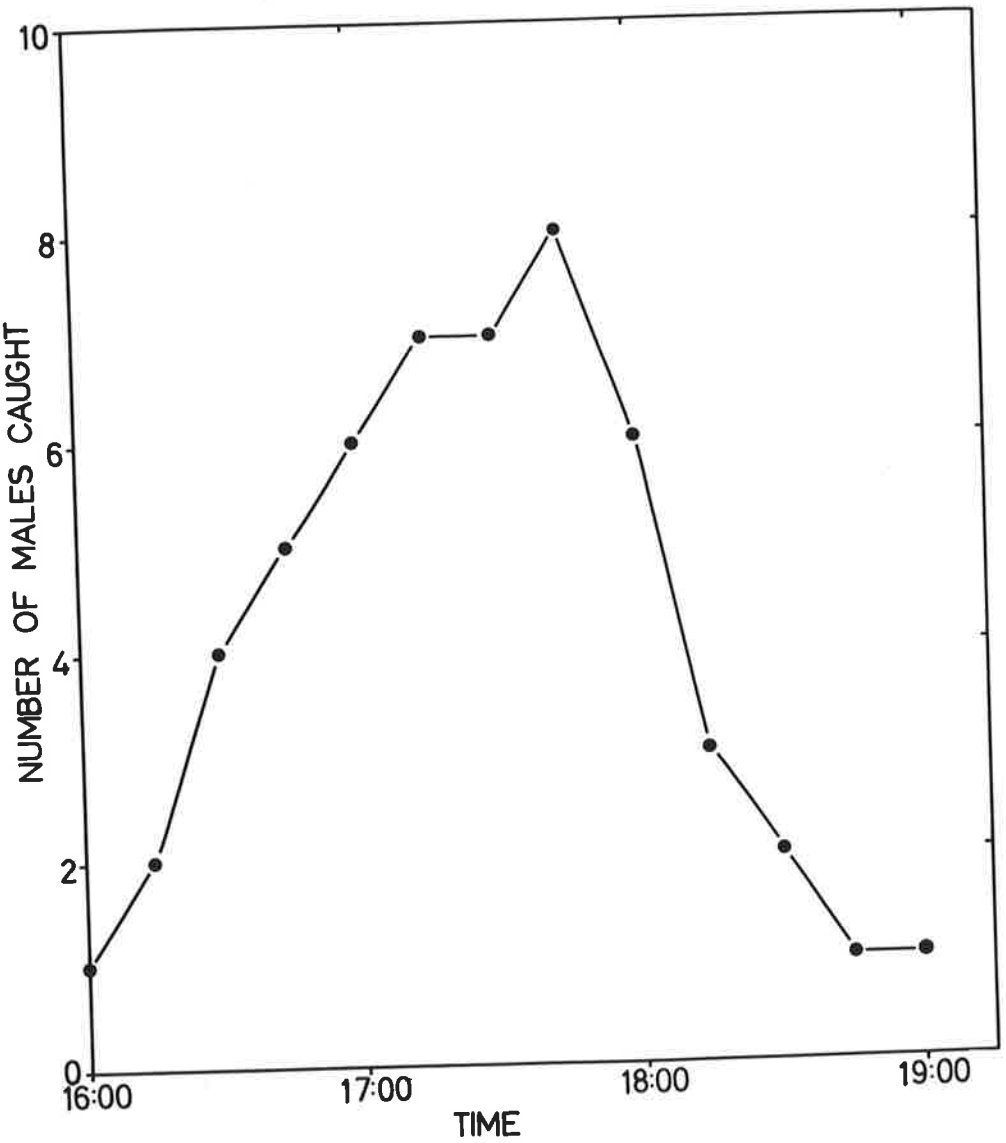
Male response was not uniform throughout the afternoon, and showed a peak between 16.00 and 18.00 h. The results indicated that tests were best conducted between 14.00 and 18.00 h, during the most active period of flight.

4.3 Conclusion and Discussion

The T-tube apparatus described here gave reliable and reproducible results when the synthetic sex pheromone of red scale was bioassayed. The main feature of this apparatus is that male red scales traverse a given distance in time when they move to the pheromone source and actually make a choice. This technique is particularly advantageous for studies of pheromone-wind interaction, because the males are allowed to travel upwind toward either the wind or the pheromone (Kennedy and Marsh, 1974; Marsh et al., 1978).

The number of males caught in sticky slide traps was increased by increasing the numbers of virgin females from 5 to 200. However, a further increase in the number of females did not improve the traps'

Fig. 4.7 Numbers of males caught by virgin females at different time intervals.


10
8
6
4
2
0
NUMBER OF MALES CAUGHT
16:00
17:00
18:00
19:00
TIME

efficiency. Rice and Moreno (1970) reported that about 50-100 pheromone-producing females/trap would be sufficient to collect maximum of responding male scales in groves with a large endemic population. However, most groves are not so heavily infested in our test. Also, some natural mortality of females would occur in the field, and some females might somehow become mated. Therefore, the attractant level per trap was initially standardized at 200. This level was used for most subsequent laboratory and field tests.

The attractiveness of females and the release of pheromone depends greatly upon their age. It has been shown by Ouye and Butt (1962) that pink bollworm females were most attractive on the third and fouth day after emergence. The virgin females of angoumois grain moth, Sitotroga cerealella (Oliver), were most attractive between 48 and 77 h after emergence (Krys and Mill, 1968). Struble and Jacobson (1970) found no detectable quantities of pheromone in the virgin females of redbacked cutworm, Euxoa chrogaster (Guenee), until they were seven days old, thereafter the concentration increased with age at least up to 20 days. However, in the case of the red scale, pheromone is released as soon as the females reach maturity. The youngest females were the most attractive and drew more than the old females.

The male responsiveness to sex pheromone exhibits a diel rhythm. Peak male activity of red scale was noticed between 16.00 and 18.00 h. After 18.00 h the activity gradually decreases until sunset (Fig. 4.6), and there is no male activity between night and midday the next day. A more detailed study (Fig. 4.7) indicated that male responsiveness increases rapidly between 16.45 and 17.45 h.

This sort of daily response could be attributed to an endogenous rhythm of sexual behaviour in the males, or it may be in response to an endogenous rhythm of female pheromone release (Baker and Carde', 1979), or it may be a combination of both factors. The length of photoperiod may play an important role in this daily rhythm response of males under natural conditions.

CHAPTER 5

STUDIES ON THE FLIGHT OF MALES ATTRACTED TO FEMALES PHEROMONE IN A WIND-TUNNEL

5.1 Introduction

After several years of rapid progress in the chemical identification of insect pheromone components, more attention is now being given to the behavioural components of pheromone action (Shorey, 1977). According to several pioneer workers on insect olfactory responses to distant odour sources (Wright, 1958; Kellogg and Wright, 1962; Farkas and Shorey, 1973; Kennedy, 1977a,b), the behavioural steps most neglected in laboratory bioassays of insect attractants are those involving free flight in moving air. Despite the proven utility of wind tunnels in such studies (Visser, 1976; Kennedy, 1977b; Miller and Roelofs, 1978; Carde' and Hagaman, 1979; Sanders et al., 1981), flight tunnels have been used only sparingly to investigate responses to pheromone. Notable, however, are studies of the mechanisms by which moths orient to and follow a plume of pheromone (Farkas and Shorey, 1972; Farkas et al., 1974; Kennedy and Marsh, 1974; Kennedy et al., 1978, 1980, 1981). Flight tunnels of varying degrees of sophistication have also been used for pheromone bioassay in which anemotactic flight has been used as a criterion (Traynier, 1968; Dahm et al., 1971; Mayer, 1973). In spite of such encouraging results, flight tunnels have yet to become popular tools in pheromone research.

In the following studies a large corridor was used as a wind tunnel in order to observe the behaviour of flying insects under as nearly natural conditions as possible. Sticky slide traps baited with virgin females reared on lemon were used to attract the males.

Fluorescent dust was used to mark males, so thet their behaviour to the female pheromone trap could be observed under ultraviolet (UV) light.

5.2 Methods and procedures

5.2.1 Wind tunnel

Experiments were carried out in a corridor (wind tunnel) 20 m long, 2 m wide and 3 m high with diffused "daylight" fluorescent lighting giving about 150 lm./ft.^{2} at floor level; the average temperature was about 23°C.

Air flow was obtained by placing a two-speed fan at one end of the tunnel, the opposite end of tunnel was open. Subsequently a flow survey through the tunnel was made at nominal wind speeds of 0.5 and 1 m/sec. Wind speed was measured with a Wallac anemometer (Wallac Oy Co., Ltd. Finland).

5.2.2 "Smoke" plume

The sharp and structure of the pheromone plumes spreading from the trap in the tunnel was obtained by releasing ammonium acetate "smoke" (generated by passing a stream of air through ammonium hydroxide and acetic acid solutions) from the position normally occupied by the pheromone source.

Three wind speeds of 0, 0.5 and 1 m/sec were used in the experiments. The plumes of smoke produced at wind speeds of 0.5 and 1 m/sec moved downwind from the source, and it was observed that the plume was broken up into irregular wisps or filaments of varying density. The filaments grew and changed shap as they were carried along the tunnel. The horizontal and vertical dimensions of the smoke plumes were similar in the two wind speeds, though with a tendency to be narrower at the high speed.

5.3 Techniques of markimg males with micronized fluorescent dust

Many methods of marking insects have been reported (Gangwere et al., 1964; Southwood, 1966; Stern and Mueller, 1968; Crumpacker, 1974), some of which seemed applicable to a wide variety of insects. Also, the methods of MacLeod and Donnell (1957) and Thomas (1951) who used either fluorescent or soluble dyes seemed suitable for small insects. Rice and Moreno (1969) reported that laboratory-reared adult males of red scale were tagged by dusting the host lemons with Calco oil blue RA dye just before emergence of the insects.

The flight behaviour of the males cannot be adequately observed with the naked eye because they are very small, about 0.6-0.8 mm in length. However, by allowing males on emergence to walk over fluorescent dust as marker, free flight and pheromone-searching behaviour of the males could be easily observed under ultra-violet light.

Pre-emergence adult males reared on lemon were placed in a blackened fiberboard carton. A filter paper tube, coated inside with fluorescent dust, was inserted into the lid of the fiberboard container. A transparent plastic vial was placed over the end of the tube. Emerging males flew to the filter paper tube, alighted on it and then walked toward the light and into the vial; they picked up particles of the dust as they walked.

Five different fluorescent dusts, Orange, Orange-Red, Orange-Yellow, Green, Blue and Optical-Whitener(American Radium Co.) were used to evaluate their effectiveness in marking the males. The results appear in Table 5.1. The Orange-Yellow fluorescent dust appeared superior in marking the males, and it also proved the most easily visible under UV light.

In order to examine whether the adult males were damaged, or unable

Table 5.1 Evaluation of various fluorescent dust in marking males of California red scale in laboratory test.

Fluorescent color (under UV)	Approx. mean particle size (μ)	Number of males emerging[a]	Number of males marked[b]	Percentage of marked
Orange-Yellow	2.0	76	52	68%
Orange	2.5	78	46	60%
Orange-Red	3.5	75	42	56%
Green	3.5	69	37	54%
Blue	4.0	71	33	46%
Optical-Whitener	5.0	72	30	42%

a) From 100 pre-emergence adult males reared on lemon.

b) The numbers of males marked were significantly different on the various fluorescent dust particles, as determined by a contingency test ($P<0.01$).

to fly, or had reduced pheromone-searching ability after they were marked, tests were conducted using a trap baited with 200 virgin females. The pheromone trap was placed at height of 1.5 meters, 4 meters away from the release carton. As indicated in Table 5.2, significant numbers of marked males were caught in the trap and most of the males were well tagged.

Rice and Moreno (1969) reported tagging of male red scale by dusting the host lemons with Calco oil blue RA dye just before emergence of the insects. The capture of tagged males can be immediately established in the field.

The usefulness of the self-marking technique described here with fluorescent dust for tagging California red scale males has been demonstrated in several laboratory experiments. It has several advantages over previously reported methods. Emerging males flew to the filter paper tube, picked up particles of the dust as they walked; this avoids direct handling of these small, fragile insect. Also, the amount of dust carried by each insect could be controlled by the filter paper tube, coated inside with a minimum of fluorescent dust; this avoids male scales pick up ~~too~~ 50 much powder that they are unable to fly. Probably the greatest utility of this method is that free flight and pheromone-searching behaviour in the marked males could be easily observed under UV light. The greatest disadvantage of the method is that after capture in traps insects need be taken back to the laboratory for processing with UV light.

5.4 Observation on the free flight and pheromone-searching behaviour of marked males

A trap baited with 200 virgin females was used as a pheromone source, and placed in the middle of the corridor 1.5 m above the floor. A cage of pre-emergence adult males was placed at the downwind end, four meters

Table 5.2 Evaluation of various fluorescent dusts in marking males, determined by free flight to a sticky slide trap baited with virgin females.

Fluorescent color (under UV)	Number of males emerging[a]	Number of males caught in trap		
		Marked	Unmarked	% of marked[b]
Orange-Yellow	73	24	19	56%
Orange	69	19	26	42%
Orange-Red	71	16	33	33%
Green	73	14	34	29%
Blue	75	11	41	21%
Optical-Whitener	68	9	37	20%

a) From 100 pre-emergence adult males reared on lemon for each test.

b) The percentages of marked males were significantly different on the various fluorescent dust particles, as determined by a contingency test ($P<0.01$).

away from the pheromone source. The emerging males walked over the fluorescent dust tube, flew from its open end and their tracks could be clearly observed under UV light.

After leaving the dust tube, a male stimulated by the pheromone would advance upwind along the central corridor towards the trap, and usually reached the source very quickly. In the absence of a pheromone source, (a trap with lemon only as a control), most males flew upwind, but the flight tracks tended to become transverse until they flew out of the observed, central region of the corridor.

5.5 Orientation in wind

Anemotaxis (orientation to an air current) has been shown to play a key role in the steering of certain flying insects to a distant odour source (Kennedy and Marsh, 1974).

Sixty marked males were released one at a time from the middle of the tunnel in wind speeds of 0, 0.5 and 1 m/sec. Records of the course and track were taken from all fliers that upwind or downwind flights along the tunnel. The numbers of upwind and downwind flights for each test are shown in Table 5.3.

Further tests showed that the distribution in nominal zero wind speed never differed significantly from 50:50; whereas the distributions in nominal wind speeds of 0.5 and 1 m/sec. always differed highly significantly from 50:50, with the majority moving upwind.

Table 5.3 Incidence of upwind and downwind flights among males passing the tunnel in various wind speeds.

Wind speed (m/s)		Total number of released males	Males flying	
Nominal	Measured		Upwind	Downwind
0	0.05(variable)	60	29	31
0.5	0.45 - 0.55	60	42	18
1	0.09 - 1.00	60	46	14

$X_2^2 = 11.58$ $P<0.001$

5.6 The effect of wind velocity and direction on pheromone trail-following by flying males

Many flying insects identify their mates and food by olfactory cues. The molecules drift on air currents away from their source, but it is doubtful that concentration of pheromone per se provides directional cues (Kennedy and Marsh, 1974). An insect detecting pheromone many meters downwind must use some mechanism to steer towards the odour source. Anemotaxis has been accepted by most investigators as the only available mechanism for the orientation of a flying insect to an odour source (Kennedy and Marsh, 1974), although (Farkas and Shorey, (1972) have proposed an alternative mechanisms based upon perception of the plume's boundary and not requiring any wind.

The effect of air velocity on anemotaxis has been examined in several species of insects (Kuenen and Baker, 1982). Field observation often indicates that the number of individuals arriving at a pheromone source decrease as the wind velocity falls below some threshold value.

In the introduced pine Sawfly, Diprion similis (Hartig), large number of males were observed flying to a source of female sex pheromone when wind velocities were above 90 cm/sec., fewer males approached the source at velocities less than this value (Casida et al., 1963). The decreased number of individuals reaching the pheromone source when air velocities were low might be attributed to an insect's inability to detect sideslippage visually, since lower wind velocities result in less relative sideslippage when the insect's flight speed and direction remain constant.

In order to estimate the effect of wind velocity and direction on male flight in the wind tunnel, two experiments were done: in experiment 1,

the pheromone source was placed upwind; in experiment 2, the pheromone source was placed downwind.

Experiment 1:

Three conditions were tested: Condition 1 - a pheromone source in moving air (1 m/sec); condition 2 - a pheromone source in still air; and condition 3 - no pheromone source in moving air (1 m/sec).

A sticky slide trap baited with 200 virgin females, or a trap with lemon only were placed at the upwind end, 1.5 meter high and 6 meters away from emerging males (100 pre-emergence adult males reared on lemon). The experiments were conducted on single individuals in the afternoon between 14.00 and 18.00 h, during the period of peak male emergence and flight activity. The results obtained for each condition are shown in Table 5.4.

The results clearly demonstrate that significant numbers of flying males were caught in the pheromone traps in moving air (condition 1) or in still air (condition 2). In the absence of pheromone, and in moving air (condition 3), few males were caught.

Experiment 2:

In this experiment, the pheromone source was placed downwind of the male. As before, three conditions were tested: condition 1 - a pheromone source in moving air (1 m/sec); condition 2 - a pheromone source in still air; and condition 3 - no pheromone source in moving air (1 m/sec). The trap baited with 200 virgin females was placed 1.5 meters and 6 meters away from the males. The results obtained for each condition are shown in Table 5.5.

Table 5.4 Number of males flying through the tunnel to the trap for the three test conditions (upwind).

Conditions	Number of males emerging	Number of males caught*
1. Virgin females on lemon; moving air	78	45
2. Virgin females on lemon; still air	71	19
3. Lemon alone; moving air	74	3

* The numbers of males caught were significantly greater in conditions 1 and 2 than in condition 3, as determined by a contingency test ($P<0.01$).

Table 5.5 Number of males flying through the tunnel to the trap for the three test conditions (downwind).

Conditions	Number of males emerging	Number of males caught*
1. Virgin females on lemon; moving air	69	11
2. Virgin females on lemon; still air	67	14
3. Lemon alone; moving air	75	1

* The numbers of males caught were significantly greater in conditions 1 and 2 than in condition 3, as determined by a contingency test ($P<0.01$).

In still air, a pheromone-permeated space was probably formed surrounding the trap ("pheromone cloud"), males were probably attracted if they entered the pheromone cloud by chance.

In the moving air, the pheromone scent was blown away to the downwind end. Males progressing downwind and passing through the source made a rather sudden turn through 180° after reaching the plume. A significant number of males was thus attracted to the lee side of the trap.

An insect that has lost the scent may made a series of across-wind casts, but also sometimes flys downwind before reorienting to the source, as suggested in Drosophila (Kellogg and Wright, 1962). In fact, under natural conditions, the odour plume is more or less filamentous and irregular because of turbulence, such turbulence might well blur the destination between an upwind and a downwind source. Evidence was in fact provided in the field trapping tests, that a significant number of flying males were caught in downwind traps.

5.7 Distance of attraction

Experiments were conducted to estimate the effect of distance in pheromone communication using various combinations of wind velocities and directions. Experiments were carried out in the tunnel using the trap baited with 200 virgin females placed at different distances from the males.

Experiment 1:

Two wind speeds, of 0.5 and 1 m/sec. were used in this experiment. One hundred pre-emergence adult males were placed downwind. Traps baited

with virgin females were placed at 4, 6, 8, 10 and 12 meters away and 1.5 meters above the floor.

Each distance was tested in the afternoon between 14.00 and 18.00 h. The results are shown in Table 5.6 and 5.7. The highest number of males was caught when the traps were placed within 8 meters from the point of male emergence. As the distance increased, the numbers of males caught decreased sharply. It is difficult to speculate on the flight range of males, but the results indicated that few males flew to traps placed 10 and 12 meters. This could have been due either to the fact that males are unable to fly long distances, or they may be less stimulated by females placed at distances of 10 meters or more. The results indicate that for each distance higher numbers of males were attracted to traps in a 1 m/sec. air flow than in 0.5 m/sec.

Experiment 2:

The experiment was repeated with the traps downwind. The same conditions were used as previously. The results are presented on Table 5.8 Table 5.9.

The data show that few males were caught when traps were placed downwind, and there were no marked differences between the number of males caught in traps in the two wind velocities.

5.8 Comparison of the effectiveness of several traps placed at different distances from a source of males

An experiment was set up to estimate the effectiveness of female traps when a number were used concurrently at different distances upwind from a source of males.

Table 5.6 Effect of distance from emergence point on number of males caught in traps baited with virgin females (wind speed 1 m/s).

Distance from the males emerging (m)	Number of males emerging[a]	Number of males caught[b]	Percentage of males caught
4	73	50	69%
6	69	46	67%
8	71	39	55%
10	76	26	34%
12	68	17	25%

a) From 100 pre-emergence adult males reared on lemon.

b) The numbers of males caught by the traps were significantly different on the various distances from the males emerging, as determined by a contingency test ($P<0.01$).

Table 5.7 Effect of distance from emergence point on number of males caught in traps baited with virgin females (wind speed 0.5 m/s).

Distance from the males emerging (m)	Number of males emerging[a]	Number of males caught[b]	Percentage of males caught
4	70	45	65%
6	72	44	61%
8	76	38	50%
10	69	21	30%
12	72	15	21%

a) From 100 pre-emergence adult males reared on lemon.

b) The numbers of males caught by the traps were significantly different on the various distances from the males emerging, as determined by a contingency test ($P<0.01$).

Table 5.8 Effect of distance on males emerging downwind of traps baited with virgin females (wind speed 1 m/s).

Distance from the males emerging (m)	Number of males emerging[a]	Number of males caught[b]	Percentage of males caught
4	71	12	17%
6	70	10	14%
8	67	7	10%
10	75	4	5%
12	68	2	3%

a) From 100 pre-emergence adult males reared on lemon.

b) The numbers of males caught by the traps were significantly different on the various distances from the males emerging, as determined by a contingency test ($P<0.05$).

Table 5.9 Effect of distance on males emerging downwind of traps baited with virgin females (wind speed 0.5 m/s).

Distance from the males emerging (m)	Number of males emerging[a]	Number of males caught[b]	Percentage of males caught
4	73	13	18%
6	69	11	16%
8	76	10	13%
10	70	6	9%
12	74	3	5%

a) From 100 pre-emergence adult males reared on lemon,

b) The numbers of males caught by the traps were not significantly different on the various distances from the males emerging, as determined by a contingency test ($P>0.05$).

Sticky slide traps baited with an equal number of virgin females, were placed 1.5 meters above the floor and 4, 6, 8, 10 and 12 meters away from a source of pre-emergence adults.

The experiments were run in the afternoons between 14.00 and 18.00 h. Two wind speeds of 0.5 and 1 m/sec. were used. The results are shown in Table 5.10 and 5.11, and indicate that the highest number of males was caught in the traps at 4 meters in both wind speeds.

If females can be considered to release pheromone simultaneously in the tunnel, the pheromone presumably accumulated and reached its highest concentration in the vicinity of the traps at the distance of 4 meters. This could well explain why the largest number of males was caught in this trap.

5.9 Conclusion and Discussion

Almost equal numbers of fliers were observed travelling each way past the tunnel in still air, and Table 5.3 indicates that a significantly greater number of males flew upwind in wind speeds of 1 and 0.5 m/sec.

It has been generally held that insects steer toward a distance odour source not chemotactically but anemotactically, by turning into the wind when they receive an odour stimulus (Kennedy and Marsh, 1974).

It has been reported, for various flying insects including *Drosophila* and *A. Kuhniella*, that, when the insect emerges from an odour plume either laterally (in a zigzag flight) or after overshooting the source (when the odour stimulus therefore ceases), the insect then turns into a crosswind track which reverses at intervals between left and right. This latter behaviour is usually referred to as "casting" or even as "searching" and is

Table 5.10 The effectiveness of pheromone traps when tested simultaneously at different distances upwind from a source of males (wind speed 1 m/s).

Distance from the emerging males[a] (m)	Number of males caught[b]
4	43a
6	20b
8	5c
10	0
12	0

a) 76 males emerged from 100 pre-emergence adult males reared on lemon.

b) Means followed by the same letter are not significantly different at the 5% level of confidence based on Duncan's multiple range test.

Table 5.11 The effectiveness of pheromone traps when tested simultaneously at different distances upwind from a source of males (wind speed 0.5 m/s).

Distance from the emerging males[a] (m)	Number of males caught[b]
4	35a
6	18b
8	6c
10	1c
12	0

a) 72 males emerged from 100 pre-emergence adult males reared on lemon.

b) Means followed by the letter are not significantly different at the 5% level of confidence based on Duncan's multiple range test.

not indentified explicitly as anemotactic.

Farkas and Shorey (1972) showed that Pectinophora gossypiella males can follow a pheromone trail in stationary air over less than 2 m, but males had taken off into the odour plume while the wind was still blowing. The possibility is therefore not excluded that their flight direction had been set anemotactically before the wind stopped and was maintained in part by visual cues.

The present results clearly demonstrate (Table 5.4) that the numbers of flying males attracted by the pheromone trap in moving air was significantly greater than in still air. In the absence of pheromone however, very few males were caught in the traps in moving air.

Some physical characteristics of the structure of the odour plume may be used by the flying males to determine the longitudinal axis of the plumes and the direction of the odour source (Farkas and Shorey, 1972). A plume in wind is not uniform but is filamentous in structure with the average molecular density being higher at the longitudinal axis than near the edges. As the male traverses at an angle across the plume, it is stimulated to repeatedly turn back toward the longitudinal axis when it encounters a certain decreased frequency of molecules in the filaments (Farkas and Shorey, 1972; Kennedy, 1977).

Kennedy et al. (1980) now proposes that persistent unwind flight requires decreases as well as increases of the pheromone stimulus and that the programmed switching of the flight track between left and right of the wind-line, seen in zig-zagging and casting flight,is not a response to loss of pheromone. It is initiated by pheromone, and then modulated by pheromone changes, the amplitude of these crosswind movements changing inversely with the strength of the pheromone stimulus.

CHAPTER 6

STUDIES ON THE FEASIBILITY OF UTILIZING THE SEX-PHEROMONE OF CALIFORNIA RED SCALE

6.1 Effect of various environmental factors on attraction of males to traps baited with virgin females

6.1.1 Introduction

Despite an increase in the use of traps to evaluate natural or synthetic sex pheromone and related chemicals, little research has been conducted to determine how the traps can best fit into the environment to obtain maximum captures of males, or of how environmental factors influence trap captures. Among the most extensive studies of this type were these of Carde et al. (1977a,b,), on the gypsy moth, Lymantria dispar (L.); Sarrio et al. (1970), on the cabbage looper moth, Trichoplusia ni (Hubner); AliNiazee and Stafford (1972), on the omnivorous leafroller moth, Platynota stultana (Walsingham); Riedl et al. (1979) on the codling moth, Cydia pomonella (L.); Bartlett et al. (1982), on the Yellow-headed spruce sawfly, Pikonema alaskensis; and Sanders (1981), on the spruce budworm, Choristoneura fumiferana (Clemens). Those authors found that the number of males captured by traps baited with living caged females or extracts of females was influenced by numerous factors, including height of the trap above the soil surface, type of surrounding vegetation and weather.

The research reported here was conducted to determine the effect of trap placement on catches of males in traps containing virgin females and to examine the influence of different air velocities and directions on the attraction of males.

6.1.2 Materials and Methods

The field work was carried out in an abandoned, heavily infested, lemon orchard in the River Torrens Gorge district of the Adelaide Hills, 25 km northeast of Adelaide. The orchard consists of a rectangular block of 260 lemon trees, ranging from 2.5 to 3.2 m high. There are no other Citrus trees and no other plants infested with red scale within a radius of six hundred meters. Also some field tests were conducted at the Waite Agricultural Research Institute.

Field trapping was conducted by using sticky slide traps. Each trap was baited with 200 virgin females on a lemon fruit. The traps were checked and randomized in the lemon fields every day. In each test an equal number of check traps (without females) was used along with baited traps. Trapping studies were conducted in December, January and February during the periods of peak red scale activity.

At the Waite Agricultural Research Institute, wind velocity and direction were recorded continuously by means of an anemobiograph (Negretti and Zambra Co.). From the records, hourly mean wind velocity (m/sec) were recorded. Continuous recordings of temperature was obtained by means of a thermohygrograph (Casella & Co.).

6.1.3 Effect of trap elevation on attraction

To test the effect of trap elevation in lemon trees, 6 baited traps, 7.5 m apart, were placed across the centre of the orchard in an east-to-west direction. The traps were suspended 0, 0.6, 1.2, 1.8, 2.4 and 3.0 m above the soil surface and their positions were randomized daily for 4 consecutive days.

The tests conducted in the lemon orchards indicated that the traps placed at 1.2 and 1.8 m attracted significantly more males (5% level), than any other higher or lower heights (Table 6.1).

6.1.4 Effect of trap distance on attraction

Experiment 1:

Six traps were placed in fallow land at 0, 4, 8, 16, 32 and 64 m from a heavily infested lemon field. The traps were hung in such a way that they were horizontal one with another, and varied in height above the soil from 1.2 - 2.8 m. Traps were placed at the appropriate distance from the edge of the orchard at intervals of 10 m, and their positions in relation to one another were randomized daily for 4 consecutive days. The daily catches were recorded.

Data shown in Table 6.2 indicate that the highest numbers of males were attracted when traps were placed in the orchard itself or within 8 m from its edge. As distance from the orchard increased the numbers of males attracted drastically decreased.

Experiment 2:

An experiment was set up in the Waite Institute grounds to estimate the effect of distance on the catches of males emerging from a single point.

Traps were placed upwind 1.5 m above the ground and 4, 8, 16, 32 and 64 m from the point where males were to be released. Two hundred emerging males were released in each test and each treatment was replicated 3 times. Because wind conditions were variable the release could only be as nearly directly downwind as possible. The results obtained, as shown in Table 6.3.

Table 6.1 Effect of trap elevation on the catches of California red scale males in the traps baited with virgin females.

Trap elevation (m)	Mean no. males attracted per trap per day*
0	6.8c
0.6	21.0b
1.2	44.3a
1.8	41.6a
2.4	26.2b
3.0	9.3c

* Means followed by the same letter are not significantly different at the 5% level of confidence based on Duncan's multiple range test.

Table 6.2 Effect of distance from infested lemon trees on catches of red scale males in traps baited with virgin females.

Distance from lemons (m)	Mean no. males attracted per trap per day*
0	33.5a
4	24.0ab
8	19.3b
16	8.6c
32	4.0c
64	2.5c

* Means followed by the same letter are not significantly different at the 5% level of confidence based on Duncan's multiple range test.

Table 6.3 Effect of distance from the release point on catches of males in traps baited with virgin females placed upwind.

Distance from the release point (m)	Mean number of males captured *
4	128.6a
8	112.0a
16	63.5b
32	28.0c
64	5.3d

* Mean followed by the same letter are not significantly different at the 5% level of confidence based on Duncan's multiple range test.

The experiment was repeated with the traps placed downwind. The same conditions were used as previously.

Results in Table 6.4 show that a low number of males were caught when traps were placed downwind from the release point. No males were caught in traps placed at 64 m.

6.1.5 The effect of wind velocity and direction on attraction

Although male red scale were caught in both "upwind" and "downwind" traps baited with virgin females, it is very doubtful that local wind directions remained invariant during the experiment. In other words, males apparently caught "downwind" might have been travelling in still air or in a local upwind current when caught. For this reason, further experiments on dispersion in relation to wind direction and velocity were conducted at the Waite Institute.

Four virgin female traps were positioned to the North, South, West and East of a release point, 4 or 8 m from the males (Fig. 6.1). Two hundred pre-adult males reared on lemon were placed in the centre of the plot. The experiment was performed in the afternoon between 14.00 and 20.00 h. Temperature, wind direction and velocity were measured during the test periods. The numbers of males attracted in each trap was counted hourly until 20.00 h (sunset 20.25 h ± 5 min).

As indicated previously (section 3.1.3), most males emerged between 17.00 and 19.00 h, and this must be taken into account when interpreting the data summarized in Table 6.5 and 6.6. Comparing catches during peak emergence in the two days, it can be seen that most males were caught upwind whatever the wind direction.

Table 6.4 Effect of distance from the release point on catches of males in traps baited with virgin females placed downwind.

Distance from the release point (m)	Mean number of males captured *
4	23.2a
8	16.0a
16	2.6c
32	0.7c
64	0

* Means followed by the same letter are not significantly different at the 5% level of confidence based on Duncan's multiple range test.

Fig. 6.1 Virgin female traps were positions to the North, South, West and East of a release point from the males.

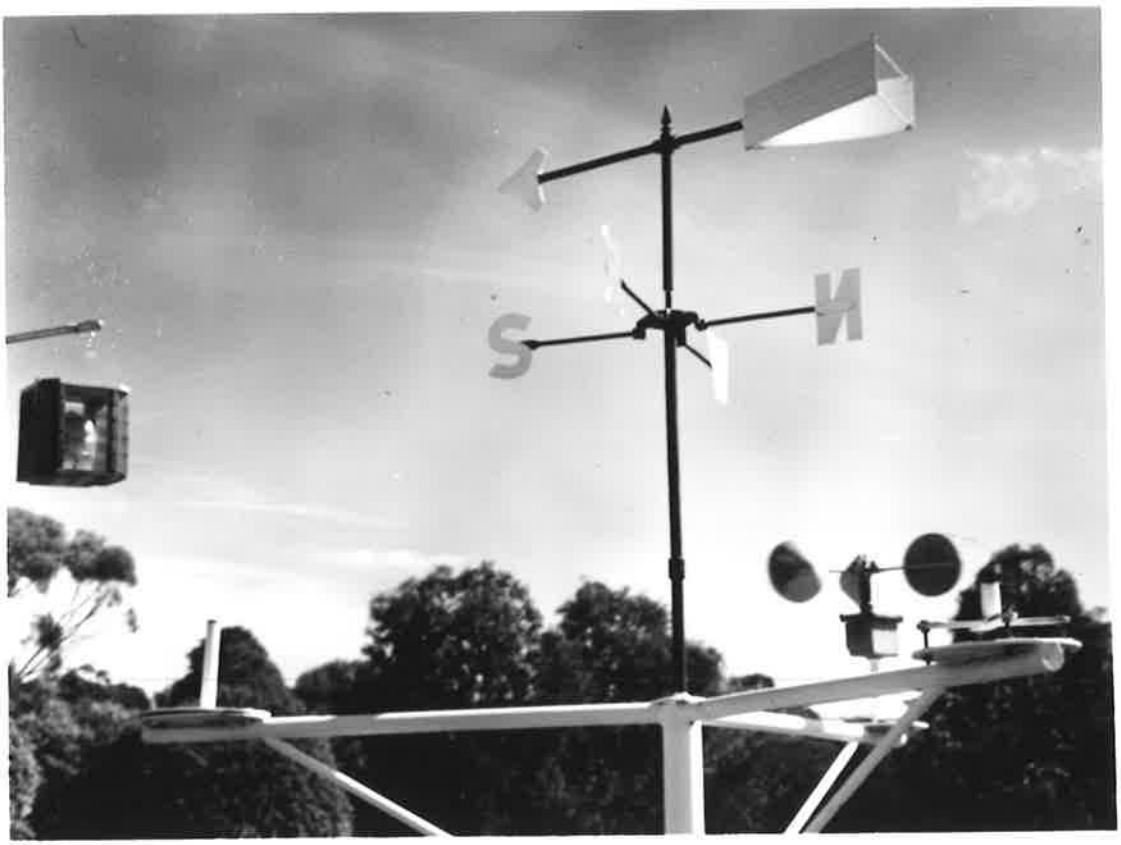

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Table 6.5 The effect of wind direction and velocity on the catches of males[a] and traps[b] baited with virgin females.

Hour counted	Number of males captured				Total	Wind direction	Wind velocity	Temp (°C)
	Trap (E)	Trap (S)	Trap (W)	Trap (N)				
14.00	0	0	0	0	0	SE	2.75	28
15.00	0	1	0	3	4	N	2.75	28
16.00	7	0	0	4	11	NE	3.00	27
17.00	11	2	1	13	27	NE	3.25	26
18.00	18	2	0	12	32	NE	3.25	26
19.00	31	0	2	1	34	E	3.50	25
20.00	12	1	0	8	21	NE	3.75	23
Total	79	6	3	41	129			

a) From a total of 158 emerging males

b) Traps placed at 4 m from the emerging males

Table 6.6 The effect of wind direction and velocity on the catches of males[a] in traps[b] baited with virgin females.

Hour counted	Number of males captured				Total	Wind direction	Wind velocity	Temp (°C)
	Trap (E)	Trap (S)	Trap (W)	Trap (N)				
14.00	0	0	0	0	0	W	3.25	28
15.00	0	0	1	5	6	N	3.50	28
16.00	1	0	4	5	10	NW	3.50	29
17.00	0	5	8	2	15	SW	3.50	28
18.00	1	23	7	0	31	S	3.75	27
19.00	8	17	0	1	26	SE	3.75	26
20.00	6	10	0	1	17	SE	3.50	24
Total	16	55	20	14	105			

a) From a total of 142 emerging males

b) Traps placed at 8 m from the emerging males

An experiment was conducted to estimate the effect of wind velocities on the attraction of males.

A trap was placed upwind at 8 m from a source of 200 emerging males. The experiment was tested in the afternoon. Wind velocities varied from 0.8 to 4.6 m/sec during the experimental period. Wind condition were highly variable, and so the numbers of males captured was recorded hourly after release.

The results obtained, as shown in Table 6.7 indicate that there was a statistically significant relationship between the numbers of males captured and wind velocity; the greater the wind velocity the more males captured.

6.1.6 Conclusion and Discussion

The placement of traps at appropriate heights has marked influence on the efficacy of sex pheromone trapping. Males of various moth species respond differently to traps place at different heights (Saario et al., 1970; AliNiazee and Stafford, 1972; Riedl et al., 1979). This may be related to the flight behaviour in relation to height of the host plant.

Statistical analysis of the data (Table 6.1) shows that a significantly greater number of flying male scales were trapped in the middle third of the trees where foliage is generally most dense and where the greatest numbers of immobile forms of red scale occur (Bodenheimer, 1951).

Data shown in Table 6.2 indicate that the highest numbers of males were attracted when the traps were placed within 8 m from males. It is difficult to speculate on the flight range of males but the data indicate that fewer males flew to the traps placed at 16, 32 and 64 m. This could

Table 6.7 The relationship between wind velocity and the capture of males in a virgin females traps.

Mean wind velocity (m/sec)	Number of males captured*
0.8	65e
1.5	96cd
2.3	108bc
3.5	129ab
4.6	143a

* Means followed by the same letter are not significantly different at the 5% level of confidence based on Duncan's multiple range test.

have been due either to the fact that males are unlikely to fly long distance in the absence of any vegetation or they may be less stimulated by females placed at a distance of 16 m or more.

The results obtained in Table 6.3 indicate a significantly higher numbers of males were caught when traps were placed upwind. However, there were still a few males caught when traps were placed downwind (Table 6.4). An insect may sometimes fly downwind and begin reorienting to a source immediately on reaching an odour plume upwind to the insect (Kellogg and Wright, 1962). In fact, under natural conditions, an odour plume is more or less filamentous and irregular, and wind direction varies considerably, and such turbulence might make it difficult to distinguish between upwind and downwind in terms of ground coordinates.

Data in Table 6.7 shown that increased wind speed resulted in an increase in the numbers of males captured. As indicated previously (section 5.6) that males of the red scales were able to respond to females at wind velocities of 1 m/sec in the wind tunnel. In the field, during peaks of male flight (late afternoon) winds were usually higher than 1 m/sec. Even in summer, wind velocities of 5 m/sec is not unusual in South Australia. So virtually all males activity and respond to females would not be inhibited by high wind velocity.

6.2 Comparison of effectiveness of lemons infested with virgin females versus synthetic pheromone

6.2.1 Introduction

Trapping experiment are commonly used to compare the behaviour activity of synthetic compounds to natural pheromone, typically emitted by caged insects. If the magnitude of trap catch in the synthetic

pheromone-baited traps matches or exceeds the catch evoked by the natural source, then the identification of the pheromone is assumed to be correct and complete with certain reservations (Carde' and Elkinton, 1984). It is assumed that both the synthetic and the natural pheromone sources emit as nearly identical rates and that the natural insects emit pheromone over the same time interval as the response to pheromone occurs. Obviously, releasing synthetic pheromone at a higher rate than natural emission potentially increases trap catch [although in some species, there may be a decrease in the performance of "late" orientation behaviour close to a stimulus at concentrations above that released by a natural source, potentially diminishing or even cancelling trap catch as in Grapholitha molesta (Busck), the oriental fruit moth (Cardé et al., 1975; Baker and Roelofs, 1981)].

It has been found that red scale females attract males with a sex pheromone. Much interest has been generated in the identification of this pheromone because no other homopteran sex pheromone has been reported and because the synthetic pheromone has immediate application in replacing the commercial virgin female traps used extensively in monitoring this pest.

Roelofs et al. (1977) identified the sex pheromone which consists of two components , 3-methyl-6-isopropenyl-9-decen-1-yl acetate and (Z)-3-methyl-6-isopropenyl-3, 9-decadien-1-yl acetate (II). A pheromone trapping system has been produced by the Zoecon Corporation, Palo Alto, California, and is now available for commercial use.

The research reported herein was a laboratory and field trapping study comparing the efficacies of virgin females vs. synthetic attractant traps for trapping the red scale males.

6.2.2 Materials and Methods

The laboratory studies were conducted in a glasshouse (5 x 4 x 3.5 m) maintained at 25-26°C. The synthetic sex pheromone cap [synthetic II (R-Z)] and pheromone® Tent ™ trap (Fig. 6.2) were supplied by the Zoecon Co.

Two types of sticky traps (tent trap and slide trap) were used for testing. The tent traps with one pheromone cap each were compared with different numbers or virgin females in the slide traps.

The field tests were conducted in the lemon orchard. The tent traps with one pheromone cap each were used along with slide traps baited with different numbers of virgin females. In another test, the tent traps with one pheromone cap each were compared with equal numbers of virgin female traps for a period of 7 days or a total of 15 trapping days at 3 different locations. Field trapping studies were conducted in December, January and February during the periods of peak red scale activity.

6.2.3 Laboratory experiment

Groups of 25, 50, 100, 150, 200 and 400 virgin females in the sticky slide traps were compared with tent traps with one pheromone cap each. Pheromone traps and female traps were hung 1.5 m high and 4 m away from the release point of the males, and 1 m apart from each other. The treatment was tested in the afternoon between 14.00 and 18.00 h, and each treatment was replicated 3 times. One hundred newly emerging males were released in each test, and all males trapped were recorded (Table 6.8).

Data from the test (Table 6.8) show that the pheromone cap trap attracted more males than the traps baited with 25, 50 and 100 virgin females, and was about as attractive as 150 virgin females.

Fig. 6.2 The synthetic sex pheromone cap and tent trap were supplied by the Zoecon Corporation.

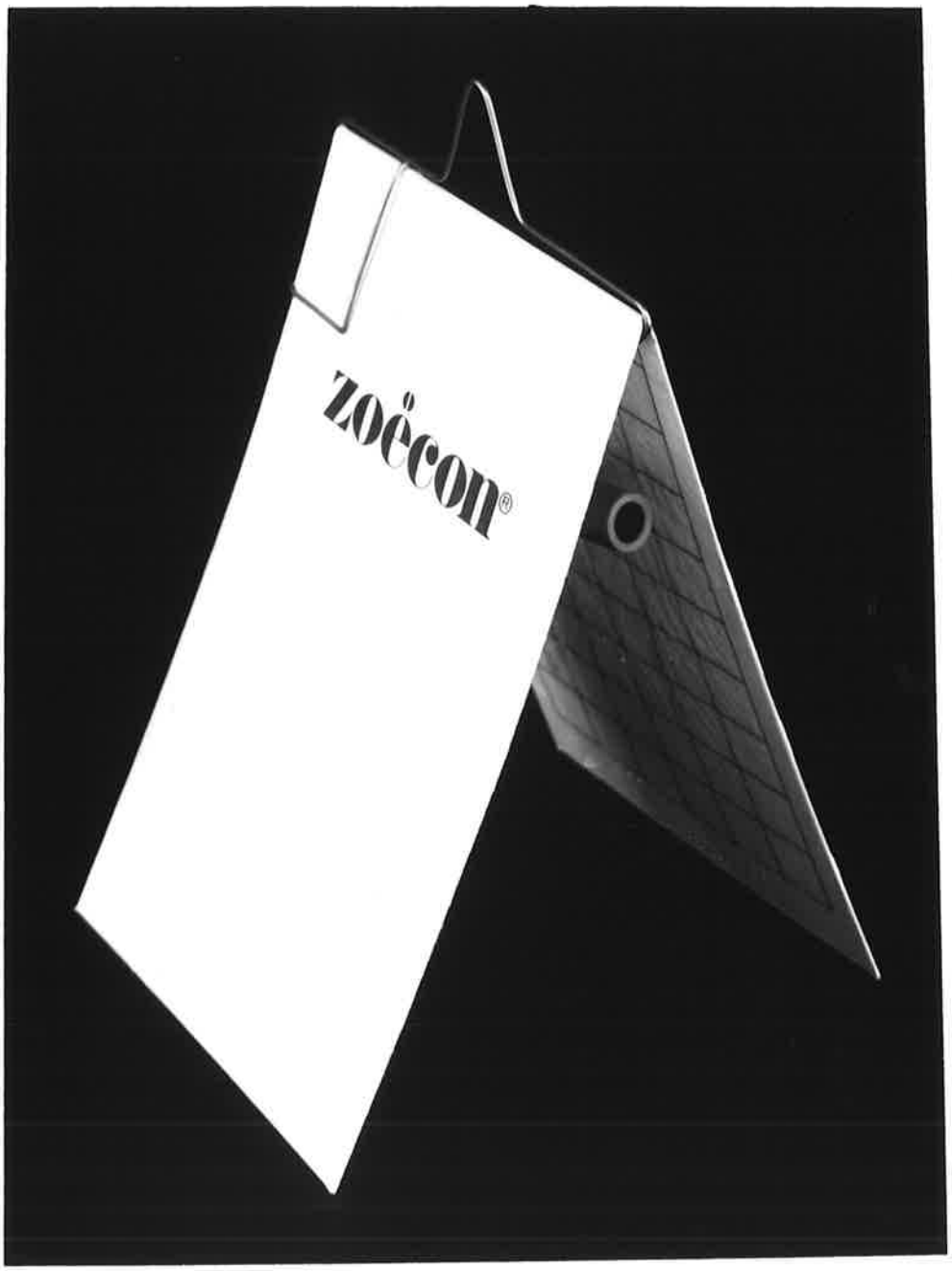

zoëcon®

Table 6.8 Comparison of the number of males attracted by the synthetic attractant traps and the females traps baited with different numbers of virgin females in the glasshouse.

Treatment	No. males captured/ per trap Mean ± SE
25 virgin females	20.7 ± 1.8
synthetic attractant	50.0 ± 4.6
50 virgin females	24.3 ± 1.8
synthetic attractant	51.3 ± 2.0
100 virgin females	33.6 ± 1.5
synthetic attractant	45.3 ± 1.5
150 virgin females	40.7 ± 2.0
synthetic attractant	41.3 ± 0.9
200 virgin females	44.7 ± 2.0
synthetic attractant	39.7 ± 1.8
400 virgin females	48.6 ± 1.9
synthetic attractant	37.3 ± 2.0

6.2.4 Field experiment

Experiment 1:

An experiment was set up to estimate the differences between the attractiveness of the pheromone traps and female traps with different numbers of virgin females in the field.

Traps were baited with 25, 50, 100, 150, 200 and 400 virgin females compared with the tent traps with one pheromone cap each.

The traps were placed in the trees in the morning and suspended 1.5 m above the ground and counts taken 24 h later for 3 consecutive days during the red scale activity. The female trap and pheromone trap were placed 1 m apart from each other, rotated and randomized each day. The results are summarized in Table 6.9.

The results indicate that the pheromone trap attracted significantly more males than those baited with 25 or 50 virgin females, and was about as attractive as 200 virgin females.

Experiment 2:

Six tent traps each with one pheromone cap were compared with six virgin female traps for a period of 7 days. The female traps used were the sticky slide traps with 200 virgin females as the attractant source. The traps were hung on the trees at a height of 1.5 m from the ground and 2 m away from the synthetic pheromone trap. The daily catches were recorded (Fig. 6.3). In another test, the female traps were compared with pheromone traps for a total of 15 trapping days at 3 different locations (Fig. 6.4).

Fig. 6.3 indicates a marked decrease in attractiveness of female

Table 6.9 Comparison of the number of males attracted by the synthetic attractant traps and female traps baited with different number of virgin females in the lemon orchard.

Treatment	No. males captured/ trap/day Mean ± SE
25 virgin females	8.0 ± 1.5
synthetic attractant	35.3 ± 1.8
50 virgin females	14.3 ± 1.5
synthetic attractant	38.0 ± 2.6
100 virgin females	22.3 ± 1.0
synthetic attractant	34.0 ± 1.7
150 virgin females	27.3 ± 3.8
synthetic attractant	31.3 ± 4.5
200 virgin females	29.7 ± 2.3
synthetic attractant	27.0 ± 1.8
400 virgin females	41.6 ± 1.8
synthetic attractant	30.3 ± 2.7

Fig. 6.3 Average numbers of males attracted by synthetic attractant and virgin female traps during 7-day trapping periods.

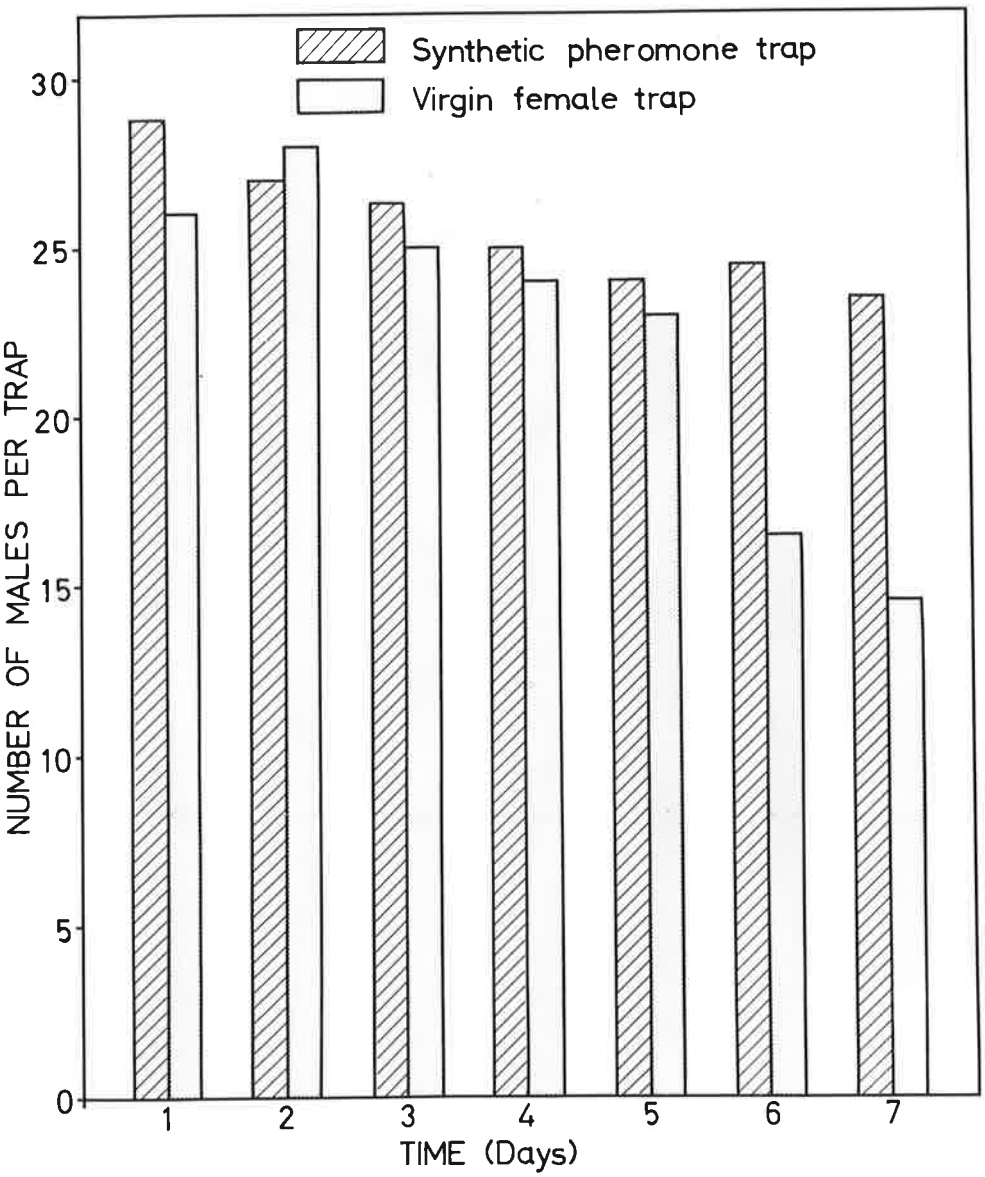
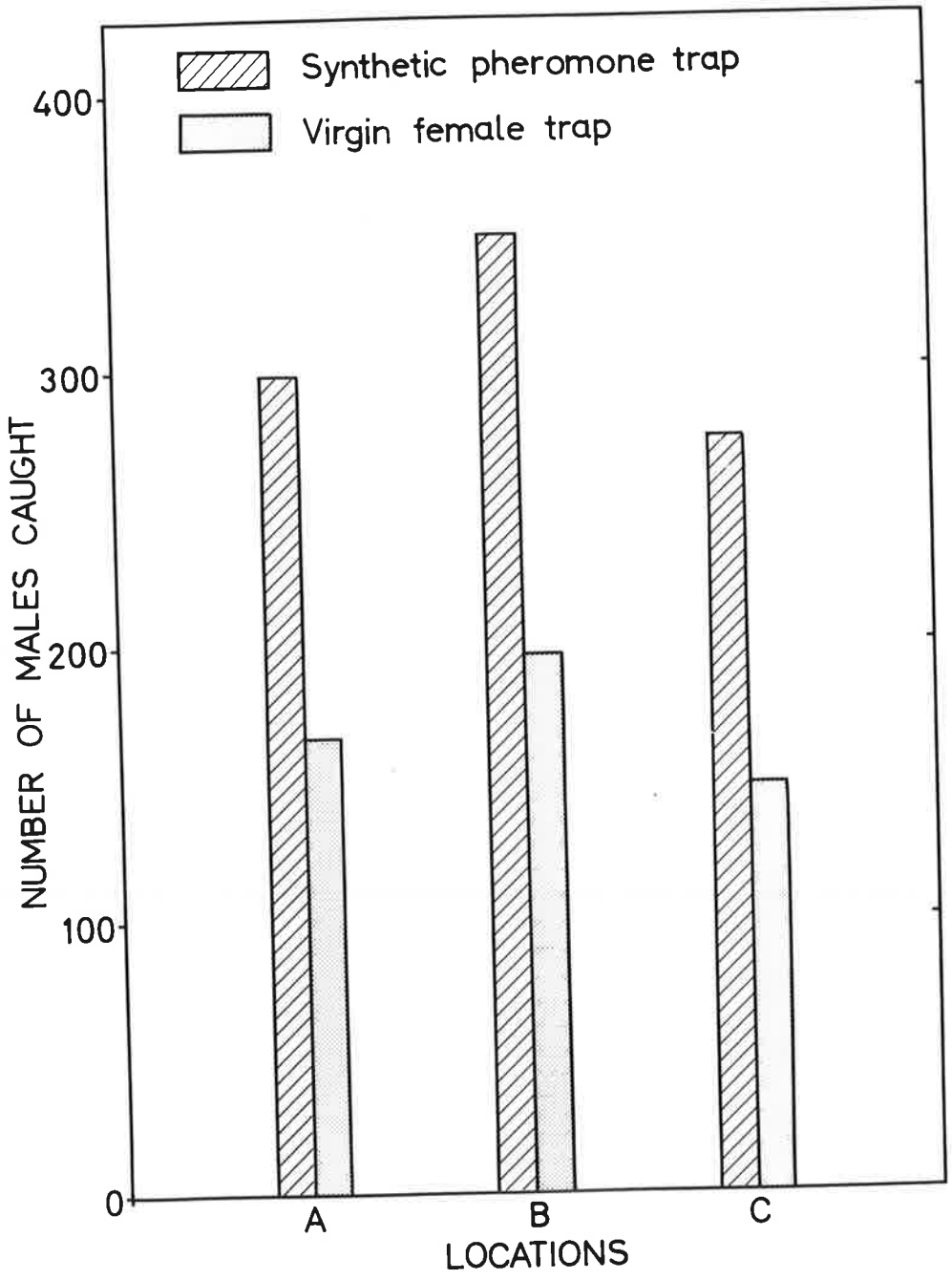

Synthetic pheromone trap
Virgin female trap
NUMBER OF MALES PER TRAP
0
5
10
15
20
25
30
1
2
3
4
5
6
7
TIME (Days)

Fig. 6.4 Total numbers of males attracted by virgin females and a synthetic attractant source during a 15-day trapping period.


Synthetic pheromone trap
Virgin female trap
400
300
200
100
0
NUMBER OF MALES CAUGHT
A
B
C
LOCATIONS

traps after the fifth day. The synthetic pheromone lures supplied by Zoecon Corporation were extremely effective. Comparison of these traps with virgin females traps for a period of 15 days (Fig. 6.4) indicates that synthetic pheromone traps were much more effective, and attracted up to twice as many males as did the virgin female traps.

6.2.5 Conclusion and Discussion

The glasshouse and field experiments indicate that the synthetic pheromone traps attracted more males than the traps baited with virgin females, and were about as attractive as 150 virgin females in the glasshouse and 200 virgin females in the field test. The results obtained in these two tests show that the synthetic pheromone attracant performed more satisfactorily than virgin females in the field.

In many insects the responder has a broader diel activity rhythm than the emitter. The synthetic pheromone was considerably more effective than a caged, emitting insect, when indeed the increased catch in a trap baited with pheromone was caused by the continuous emission of pheromone and the promiscuous sexual activity rhythm of the responders (Carde' and Elkinton, 1984).

Besides trap interactions of poaching, traps of course compete with natural pheromone emitters. The pheromone of competition has been invoked in two species, the summer fruit tortrix, Adoxophyes orana (Minks and DeJong, 1975), and the codling moth, Cydia pomonella (Howell, 1974; Riedl et al., 1979), to explain the drop in catch that occurs during the peak of female emergence. Clearly, males that are engaged in mating cannot be lured to traps, but it is not simply explicable (Minks, 1977; Carde', 1979) why trap catch should drop so precipitously unless the proportion of the calling sex is relatively high.

Another possible explanation is that traps are relatively less attractive than the calling insect. In the cases of Adoxophyes orana (Den Otter and Klijnstra, 1980) and Cydia pomonella (Bartell and Bellas, 1981) there is evidence that the entire pheromone bouquets are not yet known. It is tempting to speculate that complete pheromone blends would would restore the attractiveness of the synthetic-baited traps during the peak female emergence (Carde' and Elkinton, 1984).

In the oriental fruit moth, Grapholitha molesta (Baker et al., 1980) found no suppression of trap catch during the peak of female eclosion during the first adult flight.

The release of pheromone of females depends greatly upon their age. Fig. 6.3 indicates that virgin females release pheromone as soon as they reach maturity and the amount falls rapidly after 5 days. Also, some natural mortality of females would occur in the field. The data show a marked decrease in catch of males after the fifth day with female traps.

The applicability of red scale trapping techniques in various biological and management studies needs to be investigated. It is obvious that synthetic pheromone traps will be useful in studying population dynamics and insects behaviour and in gathering other basic information that will be needed to pursue strategies for control of red scale by pheromone trapping.

6.3 Male confusion-disruption of pheromone communication with synthetic pheromone

6.3.1 Introduction

Numerous investigators have suggested that if sufficient synthetic female sex pheromone was spread over large areas, causing the air to be

permeated to a sufficiently high level, males would be unable to locate females. To avoid implying a mechanism, this effect is termed "mating disruption" (Roelofs et al., 1979; Doane and Brooks, 1980; Carde', 1981; Rothschild, 1982; Sanders and Seabrook, 1982).

The actual means by which the airborne disruptant modifies "normal" male behaviour are little understood. Three principal hypotheses seem most appealing when the disruptant is the synthetic of the natural pheromone. These mechanisms are not mutually exclusive and could act in concert (e.g., Carde', 1981).

First, continuous or even brief exposure to pheromone may require that the concentration of pheromone requisite to elicit behaviour be elevated or pheromone responsiveness itself may be eliminated altogether. Both of these modifications could result from either sensory adaptation of peripheral receptors or habituation at a more central integrative level.

A second hypothesis proposes that males "search" normally in disruptant-treated areas and are attracted to the numerous sources of synthetic pheromone. This mechanism is particularly appealing when synthetic pheromone is released from a point source matrix such as hollow fibers, which emit pheromone at approximately the same rate as a female emite. The success of this mechanism, which may be termed the "competition" effect, clearly is dependent upon the relative attractiveness of the point sources and females as well as their ratio.

A third mechanism supposes that camouflaging the natural aerial trials by raising the concentration of synthetic pheromone sufficiently above the density emanating from the female, thereby rendering the boundaries of the natural plume indiscernible. A male in this milieu would lack sufficient

information to detect the boundaries or even the presence of natural plumes and thus to negotiate a typical zigzag course upwind to the females.

The feasibility of disrupting pheromone communication by atmospheric permeation with synthetic pheromone has now been demonstrated in the field for numerous species of Lepidoptera (Roelofs, 1979), including the Trichoplusia ni (Hubner) (Tumlinson et al., 1972; Kaae et al., 1974), Lymantria dispar (L.) (Carde' et al., 1973), Grapholitha molesta (Busck) (Gentry et al., 1975; Rothschild, 1975, 1979; Carde' et al., 1977b; Carde', 1981), Argyrotaenia velutinana (Walker) (Carde' et al., 1975; Roelofs, 1978), Choristoneura rosaceana (Harris) (Novak et al., 1978), Laspeyresia pomonella (L.) (Roelofs et al., 1972; Carde' et al., 1977), Choristoneura fumiferana (Clemens) (Sanders, 1975, 1979; Sanders et al., 1982). As a result, investigators have suggested that populations of pest insects might be controlled by using such materials to prevent orientation of male to female and thus to interrupt the normal reproductive cycle.

The experiments that follow were designed to evaluated the synthetic pheromone compound, (Z)-3-methyl-6-isopropenyl-3, 9-decadien-1-yl acetate (II) [synthetic II (R-Z)], in the laboratory and the field, as effective for disruption of the attraction of males to virgin females.

6.3.2 Materials and Methods

The laboratory studies were conducted in the glasshouse maintained at 25-26°C. Tent traps with different numbers of synthetic sex pheromone caps were tested against sticky slide traps baited with 150 virgin females. One hundred newly emerging males were released in each treatment, and each treatment was replicated 3 times.

The field experiments were conducted in the Waite Institute grounds

and in the lemon orchard. Tent traps with one pheromone cap each were used along with equal numbers of female traps with 200 virgin females in one to three concentric circles. Two hundred emerging males were released in each treatment, and each treatment was replicated 3 times.

In another test, tent traps with different numbers of pheromone caps were used along with female traps baited with 200 virgin females in the lemon orchard. Each treatment was exposed for 3 days. Field trapping studies were conducted in December, January and February during the periods of peak red scale activity.

6.3.3 Laboratory experiment

A sticky slide trap baited with 150 virgin females was hung with a tent trap with one, 2, 3 or 4 synthetic pheromone caps. The traps were 1.5 m high, and 1 m apart and 4 m away from the release point of the males. The treatment was tested in the afternoon between 14.00 and 18.00 h, and each treatment was replicated 3 times. One hundred newly emerging males were released in each test, and all males attracted in the female traps were counted.

Data from the test (Table 6.10) indicate that significantly fewer males were caught as the number of pheromone caps increased.

6.3.4 Field experiment

Experiment 1:

The experiment was conducted in the orchard to evaluate the effect of different quantities of synthetic pheromone on the attraction of males to females.

The tent traps with one, 2, 3 or 4 pheromone caps were tested against

Table 6.10 Effect of attraction of red scale males in female-baited traps placed with different numbers of synthetic pheromone in the glasshouse maintained 25-26°C.

Treatment : 150 virgin females +	No. males captured by the female traps Mean ± SE
No synthetic attractant	76.6 ± 2.3
1 synthetic attractant cap	41.3 ± 1.8
2 synthetic attractant caps	29.0 ± 4.2
3 synthetic attractant caps	21.7 ± 1.2
4 synthetic attractant caps	12.6 ± 1.5

sticky slide traps baited with 200 virgin females in pairs. A pair of traps was hung in a tree 1.5 m above ground with the traps 1 m apart (Fig. 6.5), and exposed for 3 trapping days. Captured males were counted each day and the pairs were rotated among the test trees, allowing each treatment equal opportunity at each location. Four control traps (virgin females only) were hung in separate trees for comparison.

The results shown in Table 6.11 indicate that fewer males were caught in female traps in the presence of traps with synthetic pheromone. As the quantities of synthetic pheromone increased, the disruption of males flying to female traps increased.

Experiment 2:

Trapping was done at Waite Institute in a level field. Four, 8 and 12 pheromone traps with one synthetic pheromone cap each were used along with equal numbers of female traps baited with 200 virgin females, laid out in one, 2 or 3 concentric circles, with radii of 4, 6 and 8 m (Fig. 6.6). Traps were hung 1.5 m above the ground. Two hundred emerging males were released at the centre in each treatment, and each treatment was replicated 3 times. As a control the experiment was run without synthetic pheromone traps. The results obtained, as shown in Table 6.12, indicate that significantly fewer males were caught in female traps as the numbers of synthetic pheromone traps increased.

6.3.5 Conclusion and Discussion

Laboratory and field tests indicate that males are attracted to the synthetic pheromone traps even in the presence of live virgin females. When synthetic pheromone traps were placed with female traps in 3 concentric circles, the reductions in capture of males by female baited traps was

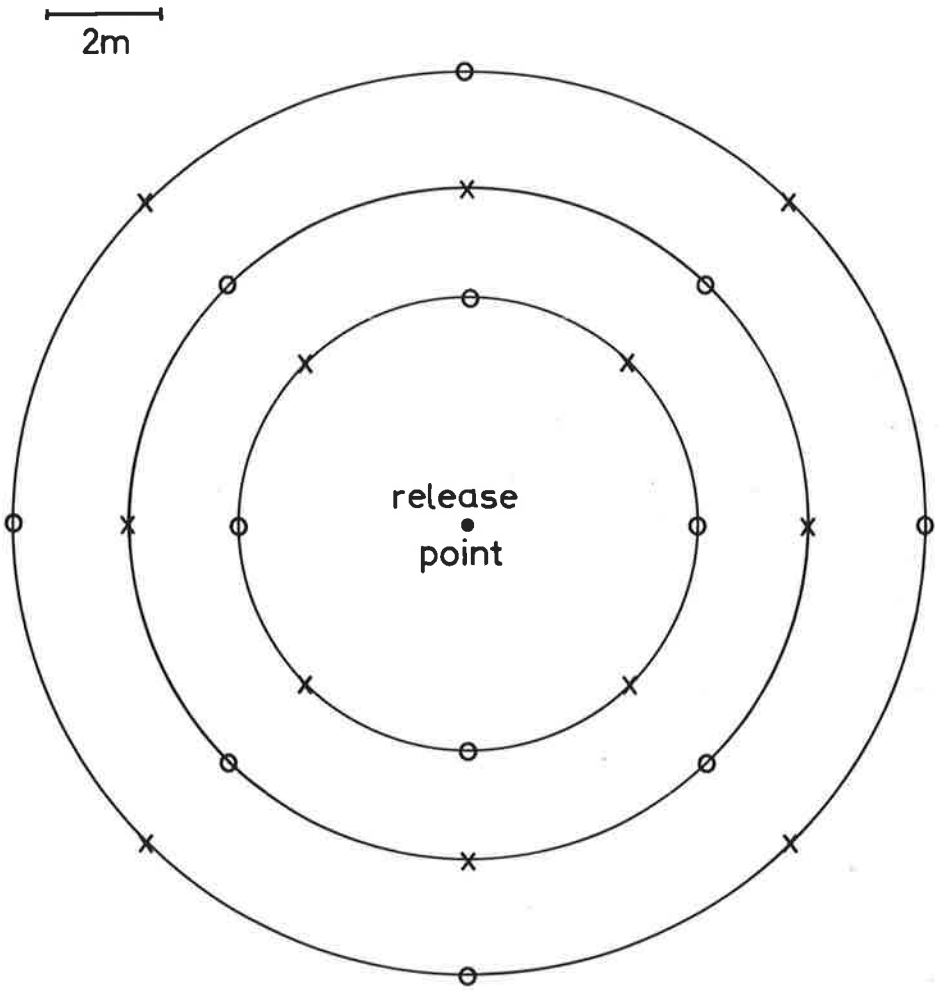
Fig. 6.5 Virgin female trap and synthetic pheromone tent trap were hung in a lemon tree 1.5 m above the ground and 1 m apart from each other.

Table 6.11 Effect of competition from synthetic pheromone traps on attraction of red scale males to female-baited traps.

Attractant	Mean no. males captured/trap/day*	
	In competition with synthetic pheromone trap	Female trap alone
1 synthetic pheromone cap	15.2a	28.3b
2 synthetic pheromone caps	11.5a	25.0b
3 synthetic pheromone caps	7.3a	30.6b
4 synthetic pheromone caps	8.6a	34.3b

* Means within rows followed by the same letter are homogenous (P = 0.05, Student's t-test).

Fig. 6.6 Four, 8 and 12 synthetic pheromone tent traps were used along with equal numbers of virgin female traps, laid out in one, 2 or 3 concentric circles, with radii of 4, 6 and 8 m.



o Female traps

x Synthetic pheromone traps

Table 6.12 Effect of competition from synthetic pheromone traps to female-baited traps in 3 concentric circles, with radii of 4, 6 and 8 m from the release point of males.

No. circles used	No. traps used	Mean no. males captured in female traps*	
		In competition with synthetic pheromone traps	Female traps alone
1	8	52.6a	116.0b
2	16	40.6a	138.3b
3	24	23.0a	154.6b

* Means within rows followed by the same letter are homogenous (P = 0.05, Student's t-test).

greater than 80% (Table 6.12).

However, because of the small area of the field experiment, it is not known whether some of males actually did orient, not to the females, but to the locally high concentration of pheromone volatilizing from the cap. More experiments will be needed in large areas to determine the minimum pheromone concentration in air necessary to prevent males from orienting to pheromone-releasing females in the field. In addition to determining minimum effective pheromone concentrations for male disruption of communication and perfecting methods for pheromone release over wide areas, several biological problems must be studied before practical behavioural control programs can be initiated. These include determination of: (1) the release rate of pheromone from normal females; (2) the threshold concentration evoking male orientation and the effective distance over which they will orient to natural emitters; (3) flight ranges and migration characteristics of males, and (4) the influence of environmental conditions on sex pheromone communication behaviour.

CHAPTER 7

GENERAL DISCUSSION

Red scale was introduced to South Australia in the early plantings of citrus near Adelaide. As early as 1897 red scale was considered a very serious pest. There have been numerous reports of outbreaks of this insect, and some of these outbreaks were so severe that insecticides had to be applied.

Life-table studies suggest that the life of the male is more hazardous than that of the females. The winged mobile males emerge from the pupae and fertilize females; some of the males were capable of mating with about 5 females. Information was also obtained on the dispersal of the alate males. Field trapping studies showed that males dispersed up to 64 meters. Rice and Moreno (1970) reported that red scale males are capable of wide dispersal in spite of their small size, fragility, and limited period of active flight; some of the males dispersed as far as 186 meters.

Emergence test conducted in the field indicated that most of the males emerged and flew in the late afternoon. Emergence was influenced by the levels of illumination and temperature. The test in the laboratory shown that when the temperature remained at 10°C or less, there was no response to light. Similarly with an increase of temperature in the absence of light emergence did not occur.

It was shown that the daily rhythm of emergence of male red scale is entrained by an interaction between the light and temperature cycles. The light is apparently the critical cue for the release of emergence, with darkness or extreme high and low temperature inhibiting it.

McLaughlin and Ashley (1977) reported that the daily rhythm of eclosion of male white peach scale, Pseudaulacaspis pentagona is entrained by the interaction of the prevailing light and temperature cycles. The eclosion rhythm is modified daily by the prevailing temperature cycle, apparently to ensure maximum survival of, and mating by the short-lived males.

Male red scale are short-lived (Tashiro and Beavers, 1968) and must quickly and efficiently locate and mate with mature females. Thus, in the field the daily synchronization of their emergence with declining temperature, usually during the afternoon or early evening, assures that they will avoid the extreme heat of the day but emerge while temperature are still high enough to sustain activity. Optimum flight temperature for male red scale is c. 27°C (Rice and Moreno, 1970), and the field activity of the yellow scale (Moreno et al., 1974) suggest that their eclosion may be regulated like that of red scale by photoperiod and temperature.

The longevity of male was longest at the lowest light intensity and temperature. Tashiro and Beavers (1968) reported that males held in darkness lived 28 h at 25°C and 42 h at 10°C. Possibly due to the reduced activity of males at lowest intensity and temperature. Survival of unmated males was significantly longer than the mated males (Table 3.9). It seems that the rate of activity of mated males slow down, while unmated ones continue to search for females.

Regulation of mating activity of red scale is affected by both light and temperature. Copulatory activity of males was found to increase with light intensity and temperature. In the field, temperature usually falls as the light intensity falls and few males copulated at temperature below about 15°C. Even in summer, 15°C is not unusual at sundown. So virtually

all seasonal activity would be inhibited by both low temperature and darkness.

Observations reported here (Table 3.12) showed that male is capable of mating with up to 5 times, contrary to Bodenheimer's report that males mate only once. No male was observed to mate with more than 5 females, but this may have been due to the effect of male age or environmental factors.

The longevity of males is very short (average 6-7 h), and copulatory activity must occur soon after emergence. Quayle (1911) stated that copulation may occur within ½-1 h after emergence. The results shown in Fig. 4.3 indicate that the male response to sex attractant was altered by their age. It was shown that one and two-hour-old males were very attracted to virgin females, whereas the older males were less so.

The attractiveness of females and the release of pheromone also depends greatly upon their age. Pheromone of red scale is released as soon as the females reach maturity, the youngest were the most attractive and drew more than twice as many males as the 2-week-old females. Females become unattractive to males shortly after insemination.

The results shown in Table 4.4 indicate that in the laboratory tests there was little difference between the attractiveness of 200 and 400 females. However, increasing the number of virgin females from 5 to 200 attracted more males. Rice and Moreno (1970) stated that about 50-100 pheromone-producing females per trap would be sufficient to collect maximum numbers of responding male scales in groves with a large endemic population. However, most groves are not so heavily infested in field. Also, some natural mortality of females would occur in the field, and some females might somehow become mated. Therefore, the optimum number of virgin female scales for use in pheromone-baited field and laboratory traps was 200.

The usefulness of self-marking technique with fluorescent dust for tagging California red scale males has been demonstrated in several laboratory experiments. It has several advantages over previously reported methods (MacLeod and Donnell, 1957; Rice and Moreno, 1969). Emerging males flew to the filter paper tube, picked up particles of the dust as they walked, avoids direct handling of these small, fragile insect. Also, the amount of dust carried by each insect could be controlled by the filter paper tube, coated inside with a minimum of fluorescent dust, avoids male scales pick up too much powder that they are unable to fly.

The effect of wind speeds of 0, 0.5 and 1 m/sec. on male behaviour was determined in the wind tunnel. Observation showed that in nominal wind speeds of 0.5 and 1 m/sec, many more males flew upwind, whereas at a nominal zero wind speed, there was no significant preference for either direction.

Responding males of red scale were found to be stimulated by the pheromone to orient upwind and to fly towards the source. The present results clearly demonstrate (Table 5.4) that in a wind tunnel the number of flying males attracted by a pheromone trap in moving air was significantly greater than in still air. In the absence of pheromone, however, very few males were caught. When the pheromone source was placed downwind of the males, the pheromone scent was blown away to the downwind end. Males progressing downwind and passing through the source made a rather sudden turn through 180° after reaching the plume. A significant number of males was thus attracted to the lee side of the trap.

It has been proposed (Farkas and Shorey, 1974; Kennedy and Marsh, 1974; Kennedy, 1977; Marsh and Kennedy, 1978) that a flying male moth finds its way to a "calling" female or other small, distant source of

wind-borne sex pheromone using two contrasting anemotactic manoeuvres: (1) upwind flight in response to the onset or increase of the pheromone stimulus, maintained as long as the stimulus conditions, and (2) cross-wind flight with switching between left and right of the wind line (the so-called track reversals (Marsh and Kennedy, 1978) or tack reversals (Carde' and Hagaman, 1979) seen in casting or zig-zagging) which occurs only in response to the loss or decrease of the pheromone stimulus. A simpler system has been proposed (Kennedy et al., 1980) in the light of wind-tunnel experiments on male summer fruit tortrix moths (Adoxophyes orana) entering homogeneous pheromone clouds. The moths did not fly persistently upwind with continuous pheromone stimulation, and their programmed left-right track reversals occurred in response to pheromone onset, not loss, and continued after pheromone loss but with widening cross-wind excursions between reversals.

Kaae and Shorey (1972) noted that in the laboratory, females of another Noctuid, Trichoplusia ni (Hubner), called for twice as long when exposed to air currents of 0.3 - 1.0 m/sec. than did females in calm air. At higher wind velocities, the length of the calling period was reduced.

The number of red scale males captured at a trap was very low at the lowest wind velocity recorded (0.8 m/sec.). At high velocities no falling off in the number of males caught was noted as the wind velocity approached the maximum recorded (4.6 m/sec.). The experiments with Trichoplusia ni, which showed a reduction in catches at greater than optimum are not strictly comparable with experiments reported here; nevertheless it may be noted that Campion et al. (1974) reported no reduction in the number of S. littoralis males arriving at the female trap as wind speeds approached the maximum recorded (5.2 m/sec.) in field trapping.

The length of time the females of red scale call at different wind velocities could well be an important factor influencing the number of males arriving at the trap and would explain the observed relationship between wind velocity and male arrivals. It is still possible that calling falls off at wind velocities higher than tested here, however, according to Minks and Noordink (1971) the arrival of the male tortrix moth Adoxophyes orana (Fisch. V. Roesl) at a pheromone source was inhibited at wind velocities of 7 m/sec. and above.

Traps placed upwind attracted more males than traps placed downwind. A larger number of males was attracted to the traps placed at 4 and 8 meters from their release point than at any other distance in the field. Sower (1973) stated that the potential distance over which pheromone communication between individual insects can occur is determined by several interacting factors. For any given wind velocity, the maximum communication distance can be estimated if the investigators know: (1) the rate of pheromone release; (2) the responder's threshold and (3) dispersion pattern of pheromone in moving air. It is apparent that communication distance will increase with increasing release rate from the emitter and increasing sensitivity (lower threshold) of the responder.

Successful pheromone communication among insects requires not only that pheromone molecules be carried by moving air to the vicinity of the responder, but also that, by appropriate orientation and location reactions, the responder moves to the vicinity of the emitter while the uninterrupted pheromone emission is taking place (Shorey, 1973).

The placement of traps at appropriate heights has marked influence on the efficacy of sex pheromone trapping. Males of various moth species respond differently to traps placed at different heights (Saario et al., 1970;

AliNazee and Stafford, 1972; Riedl et al., 1979). Statistical analysis of the data (Table 6.1) shows that a significantly greater number of flying male scales were trapped in the middle third of the trees where foliage is generally most dense and where the greatest numbers of immobile forms of red scale occur (Bodenheimer, 1951).

The results obtained in Table 6.3 indicate a significantly higher numbers of males were caught when traps were placed upwind. However, there were still a few males caught when traps were placed downwind. An insect may sometimes fly downwind and begin reorienting to a source immediately on reaching an odour plume upwind to the insect (Kellogg and Wright, 1962). In fact, under natural conditions, an odour plume is more or less filamentous and irregular, and wind direction varies considerably, and such turbulence might make it difficult to distinguish between upwind and downwind in terms of ground coordinates.

Glasshouse and field experiments reported here indicated that synthetic pheromone traps attracted more males than traps baited with virgin females, and that a single "lure" of synthetic pheromone was about as attractive as 150 virgin females in the glasshouse and 200 virgin females in the field tests. The fact that the synthetic pheromone was even more successful in competition with virgin females in the field than in the laboratory was presumably attributable to environmental factors, such as temperature and air velocity, that influence pheromone release by females.

The synthetic pheromone lures supplied by Zoecon Corporation were extremely effective. The rate of pheromone release from these dispensers was maintained by a slow release that kept the traps effective for over two weeks. Comparison of these traps with virgin female traps for a period of 15 days (Fig. 6.2) indicates that synthetic pheromone traps

were much more effective, and attracted up to twice as many males as did the virgin female traps.

In many insect, the responder has a broader diel activity rhythm than the emitter. The synthetic pheromone was considerably more effective than a caged emitting insect, when indeed the increased catch in a trap baited with pheromone was caused by the continuous emission of pheromone and the promiscuous sexual activity rhythm of the responders (Carde' and Elkinton, 1984).

The number of male scales caught on pheromone traps in each flight period during the fruit growing season can provide a means of more accurately and simply measuring population densities. It can provide a basis for estimating fruit infestation and improve the quality of decision-making in crop protection systems. However, caution is needed in the use of the trap because of the male's sensitivity to cold temperature, high wind, pesticide applications for other pests in the same orchard, pesticide drift from neighboring orchards, trap placement in relation to neighboring orchards, and quality of pheromone used (Moreno and Kennett, 1985).

Recent research has demonstrated that pheromone communication between the sexes of several species of Lepidoptera can be disrupted by permeating the air with synthetic compounds (Roelofs et al., 1979; Doane and Brooks, 1980; Carde', 1981; Rothschild, 1982; Sanders and Seabrook, 1982). It is possible that the control of red scale, could be based on the release of sufficient synthetic female sex pheromone spread over large areas to disrupt of male attraction to female. Particularly because red scale are very

short-lived, they would not be able to find and inseminate the females before they died.

Laboratory and field experiments indicated that significantly fewer males were caught in female traps in the presence of traps with synthetic pheromone. As the quantities of synthetic pheromone increased, the proportion of males flying to the female traps decreased.

Disruption of communication between the sexes to prevent or reduce successful matings may therefore be possible. Synthetic attractants may be used lures or baits incorporated with insecticides, or with sticky materials to capture and kill scale pests. The problems to be solved before the potential for their technique can be assessed are basically concerned with learning the appropriate techniques of application.

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