VISUAL BEHAVIOUR OF THE WHITEFLY *TRIALEURODES VAPORARIORUM* (WESTWOOD) (HOMOPTERA : ALEYRODIDAE)

by

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A thesis submitted for the degree of Doctor of Philosophy
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March 1981

Errata

Monochrometer in Figs. 3.1, 4.1 and 5.1 should read monochromator.

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SUMMARY

The greenhouse whitefly *Trialeurodes vaporariorum* is a pest of glasshouse crops and ornamentals and is of world-wide distribution. Previous studies on selection of food-plants by this insect have indicated that the initial processes were probably governed by visual stimuli, particularly colour, but the results are difficult to interpret because the role of ultra-violet light has been neglected and the intensities and spectral composition of the visual stimuli were not sufficiently controlled. For these reasons this project examined several aspects of the visual behaviour of the whitefly with particular emphasis on wavelength specific behaviour and colour vision.

A spectral efficiency function of "slow" phototaxis showed a peak at 400nm which contrasted with a spectral efficiency function published previously, which peaked at 550nm, and closely followed the transmission spectrum of a tobacco leaf (a favourable food-plant). This difference was explained by an examination of other aspects of behaviour, namely, flight, landing, take-off and walking behaviour at two standard wavelengths (400nm and 550nm) at a fixed quantum flux level. "Slow" phototaxis, when compared with other responses measured previously, had a different spectral efficiency curve, probably because it is composed of a different set of behavioural responses with different spectral sensitivities. Whiteflies walked faster towards the 400nm monochromatic light, oriented towards the 400nm light and took off more readily when illuminated by the 400nm light. Thus "slow" phototaxis was greater towards 400nm because this response mainly consisted of walking behaviour, whereas other

responses measured previously were composed of flight, landing and take-off behaviour. A "fall reflex", which is probably a prelude to landing, occurred in response to 550nm but not to 400nm.

A "settling" response paradigm was developed which involved the same sorts of behavioural responses involved in food-plant selection, namely, landing after a short flight. Intensity response curves had different shapes at different wavelengths, which indicated that the whiteflies were probably exhibiting wavelength specific behaviour. This was verified by modifying the behavioural paradigm to favour flight towards an illuminated surface. This measure had a different spectral sensitivity and consequently the original "settling" response paradigm measured at least two different behavioural patterns with differing spectral sensitivities.

Conditioning the whiteflies to visual stimuli using shaking as an aversive conditioning stimulus was not successful. However, using the same method, which probably measured "fast" phototaxis and involved measuring the number of insects in the illuminated half of a cylindrical container, the whiteflies clearly exhibited wavelength specific behaviour. Intensity response curves had different signs above threshold. There were no colour contrast effects and whiteflies presented with a constant high intensity of 400nm light on one side of the container and various intensities of 550nm light on the same and/or opposite side of the container, behaved as if there were no interactions of photoreceptor outputs in the central nervous system. Thus there was no evidence for colour vision at least for that particular behavioural paradigm. The "fast" phototactic paradigm at 400nm and 550nm probably measured different and antagonistic behavioural patterns.

The compound eye of *T. vaporariorum* is divided into two halves, a ventral half and a dorsal half. The ventral half has yellow corneal filters arranged in a hexagonal pattern around a clear facet, whereas the dorsal half has clear facets. The possible role of these structures is discussed.

DECLARATION

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

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Peter Eric Coombe

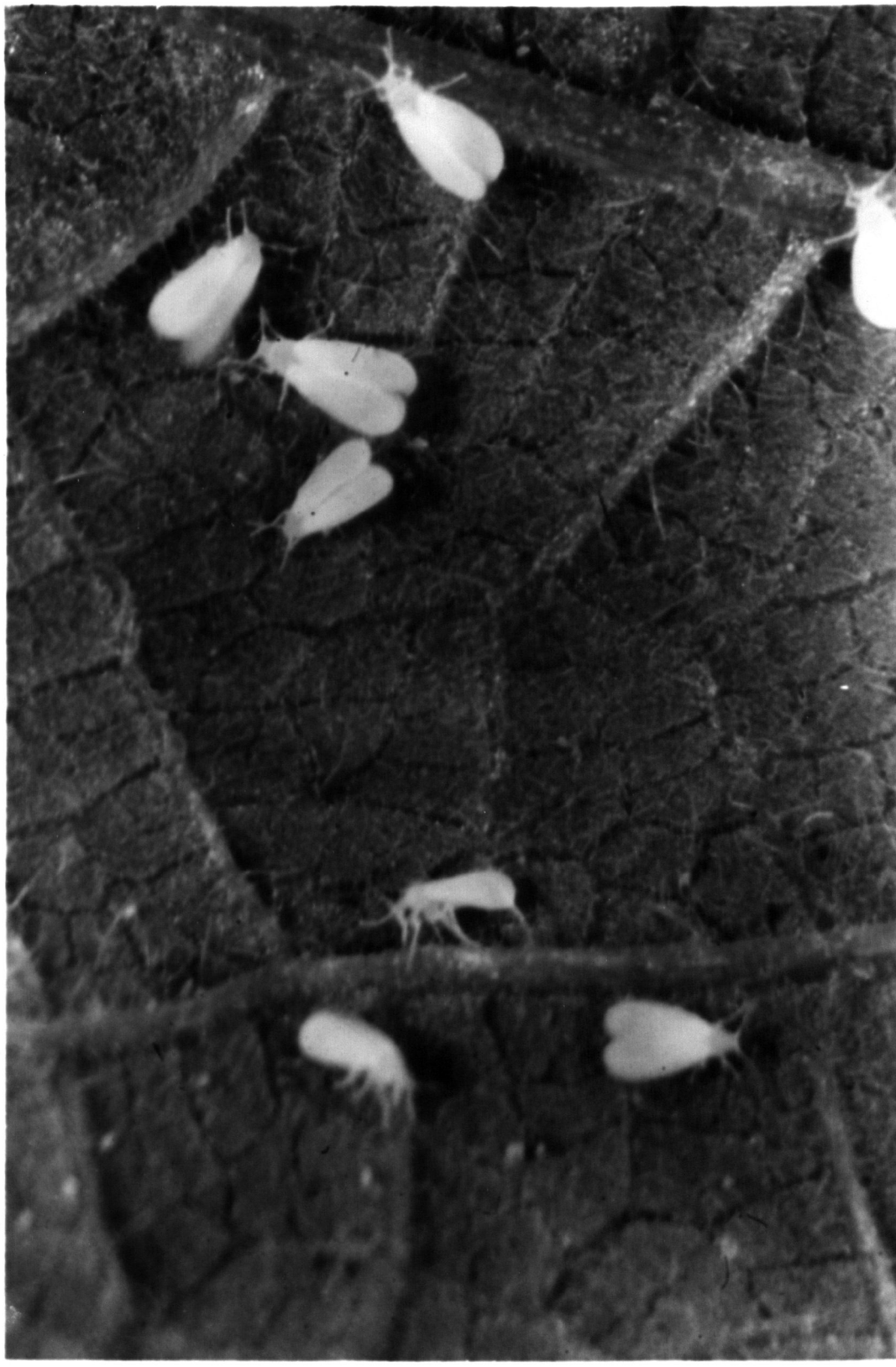
ACKNOWLEDGEMENTS

I thank my supervisor Professor T.O. Browning for many helpful discussions and friendly advice. I am also much indebted to Dr P. Miles and Dr Simon Laughlin who read drafts of the thesis and made valuable comments. I am especially grateful to Simon for the long discussions we had when I visited Canberra and during his stay in Adelaide, and also for introducing me to the Neurobiology Department at A.N.U. I would also like to thank Professor Dr Randolf Menzel for an invaluable discussion one evening in Canberra and also for his answers to my queries some twelve months later. Prof. Menzel's advice was invaluable at a critical time. Dr K.F. Fischbach has also been most helpful in answering some of my queries about his work on *Drosophila*.

One monochromator was kindly provided by the Physics Department, University of Adelaide and the other by Flinders University when the one from Adelaide was unavailable. The thermopile and milli-microvoltmeter were provided by the Physics Department, University of Adelaide. I am most grateful to Dr B. Horton for helpful technical advice in the operation of this equipment. I would also like to thank Dr Aspinall from the Plant Physiology Department for the use of his interference filters.

Others who need to be thanked are Mrs Lever for the use of an I.B.M. typewriter during the writing up period, the library for innumerable inter-library loans, Miss T. Siekmann for typing the thesis, and lastly, but certainly not least, Heloisa for many helpful discussions during the experiments, for looking after my whitefly culture when I was away, and for help in proof reading.

Plate 1 Adult whiteflies on a bean leaf. (x 20)



CHAPTER 1

1. INTRODUCTION

The greenhouse or glasshouse whitefly *Trialeurodes vaporariorum* (Westwood) (Homoptera : Aleyrodidae) (see Plate 1) is an important pest of vegetables, ornamentals and other plants in glasshouses and in the open. Food plants and the distribution of *T. vaporariorum* have been compiled by Russell (1963, 1977) and Mound and Halsey (1978). The insect is polyphagous with a large range of food plants belonging to at least 249 genera (Russell, 1977) and has a wide distribution. Direct damage to crops occurs through the feeding of large numbers of the insect, and indirect damage occurs through the growth of a sooty mould (*Cladosporium sphaerospermum*) on the honeydew excreted by all stages, especially the later instars. The growth of sooty mould reduces photosynthesis and hinders respiration, but does not reduce yields (of tomatoes) until the insects are in large numbers. More importantly, the presence of sooty mould necessitates expensive washing of fruit before sale (Hussey *et al.*, 1958). In addition *T. vaporariorum* is also a vector of a number of virus diseases, namely beet-pseudo-yellows virus in California (Duffus, 1965), a sunflower virus in Argentina (Costa, 1969) and a tomato virus in Mexico (Hernandez, 1973).

In Australia and in many other parts of the world, *T. vaporariorum* is attacked by a small chalcid wasp *Encarsia formosa* Gahan. This parasite has been used in biological control programs in a number of countries (reviewed by Vet *et al.*, 1980). In Australia, *T. vaporariorum* was a pest of tomatoes and beans and in the open prior

to 1935-36 (Noble, 1938; Anon., 1933; New South Wales Dept. Ag., 1938; Wilson, 1960). After a number of unsuccessful importations by the Commonwealth Scientific and Industrial Research Organisation (C.S.I.R.O.), *Encarsia formosa* was successfully introduced to Canberra from New Zealand in 1935, and thereafter it quickly became established. Supplies were sent to the various departments of agriculture in 1936, and by 1939 the parasite was present all over Australia (C.S.I.R.O. reports 1932-39).

Since 1936-39, *T. vaporariorum* has been notably scarce. The New South Wales Department of Agriculture pests survey mentions *T. vaporariorum* only four times from 1947-1963, three of which were as a minor pest only. In South Australia the same sort of pattern has occurred (Waite Agricultural Research Institute report 1937-38, Edwards, 1951). Edwards (1951) stated in relation to South Australia -

> "At present no control measures are necessary. Rarely is the whitefly seen in this state. *Encarsia formosa* a wasp which parasitizes the young scales under the leaves, has given such complete control that it is now difficult to find either the whitefly or the wasp."

However, from about 1971-72 *T. vaporariorum* has appeared in large numbers, especially in backyard gardens in Sydney, Melbourne, Hobart, Adelaide and Perth (Tas. Dept. Ag. Insect Pests Leaflet, Aust. Dept. Ag. New and Unusual Insect Records) but the parasite is still common and apparently causing high rates of parasitism. In order to determine whether the whitefly or its parasite had changed,

a previous study (Coombe, 1976) examined various biological factors and compared them with previously published information. It was found that the biological parameters of the whitefly that were examined, had similar values to those published previously. In particular, when the interaction of *T. vaporariorum* and *E. formosa* was investigated under controlled conditions, it appeared that the parasite was capable of controlling the whitefly under conditions that could be expected to favour the parasite. On the basis of these results it was concluded that the recent increase in the numbers of whiteflies in Australia was probably due to aberrations of the weather.

Some possibilities that were not investigated were variation of food-plant selection by the whitefly and host selection of the parasite. Since a change may have occurred in one of these, I decided to investigate firstly food plant selection of *T. vaporariorum*, because some information was available from the literature (see below), and later host selection in *Encarsia*, an area where very little work had been done. The work on food-plant selection led me into other aspects of the insect's biology however, and this left insufficient time to attempt investigation of host selection of *Encarsia*.

Food-plant selection is a catenary process (Thorsteinson, 1960). It involves a chain of responses beginning with orientation to the plant from a distance by olfactory or visual stimuli. Once landed on a plant, other stimuli such as tactile and gustatory stimuli, in addition to visual and olfactory stimuli, determine whether an insect takes off again or settles and feeds or oviposits (Beck, 1965). Vaishampayan *et al.* (1975b) has shown that the initiation of food-plant

selection in *T. vaporariorum* is probably mostly due to visual responses. When only visual cues were available, however, whitefly adults landed to an equal extent on leaves of a non food-plant and favourable food-plant. Nevertheless, the amount of time spent after landing on a non food-plant (e.g. corn) was small compared with the time spent on a favourable food-plant (e.g. bean). In addition, a small but significant preference for bean leaves over a moist filter paper was found in an olfactometer (Vaishampayan *et al.*, 1975b), but this response was not nearly as pronounced as the response to various colours (Vaishampayan *et al.*, 1975a, b). Thus odour probably plays at least a minor role in the first steps of selection of food plants by *T. vaporariorum*.

Because visual responses probably are important in selection of food plants by *T. vaporariorum*, and because colour vision may well play an important role, the aim of this study was to further elucidate the behaviour of *T. vaporariorum* to various wavelengths of light, comparing it with previously published information, and to attempt to determine whether *T. vaporariorum* had "true" colour vision.

CHAPTER 2

2. PRELIMINARIES

2.1 The Culture Method

The experiments carried out during the course of this work required large numbers of newly emerged adult whiteflies daily. The design of the culture method was based on Scopes and Biggerstaff's (1971) mass-rearing techniques for *Trialeurodes vaporariorum*. The food-plant was the French bean *Phaseolus vulgaris* variety Hawksbury Wonder. This species was chosen because of its fast growth rate, large leaves and the fact that *T. vaporariorum* is found in greatest numbers on it in backyard gardens in South Australia.

A batch of seed was planted once a week in vermiculite and after germination, transferred into sterilized soil in small pots. Two weeks after planting, the apical shoot was removed, leaving only the first two leaves, and the plants were placed in a cage into which adult whiteflies were released. One week later the plants were removed from the cage and the adult whiteflies washed off with a jet of water. This technique does not affect the eggs or the larval stages of *T. vaporariorum* but removed adults and any mites or aphids which may have found their way into the culture. The plants were left in the bulk culture for a further 2-2.5 weeks for larval development after which they were trimmed and placed in 30cm cubic cages, separate from the bulk culture, in a growth cabinet maintained at 21^{o} ($\pm$ 1^{o}C) 15L : 9D. New leaves, after the initial infestation, were kept reasonably free of whiteflies and so only a proportion of each plant supported a population

of whitefly larvae. This was essential to maintain the health of the plants. Healthy plants were important, not only to ensure consistency in the rearing conditions of the experimental insects, but also to maintain survival of the whitefly larvae. The larval stages of *T. vaporariorum* are sessile and cannot be transferred from one plant to another. The bulk culture was maintained in an insectary under artificial lighting with the temperature varying from 20^{o}-30^{o}C in winter and 23^{o}-30^{o}C in summer. At least once a year, adult whiteflies used for the initial infestations were collected from the field, rather than from plants in the culture, and so over a period of four weeks the entire culture was replaced with field insects. This was done to reduce the probability of a "laboratory strain" being produced as a result of rearing the insects over a large number of generations under artificial conditions.

Occasionally the plants were watered with a solution of pyrethrum to control a small sciarid fly, the larvae of which attack the roots of beans. Once a week all plants were watered with a solution of "aquasol" and at all times rain water was used for watering because Adelaide tap water produced symptoms of salt stress.

The culture technique described above is capable of producing a continuous supply of up to 3,000 adult whiteflies per day from ten healthy plants each week. The method has the advantage that any pest infestation can be eliminated easily because there is a continuous flow of plants. All plants are used once only and then discarded. Since plants start from seed within the culture, there is little possibility of accidental introduction of pests from outside nurseries. The main

problem encountered has been the estimation of the optimum level of initial infestation of whiteflies. If the number of whiteflies is too high, the plants either die or show severe signs of stress, but if it is too low, not enough insects are produced for experimental purposes. Any method of measuring the level of infestation is impractical because it is inevitably time-consuming since it involves counting large numbers of insects. Thus the level of infestation was chosen by an educated guess based on experience which occasionally failed.

2.2 Emergence of Adult *Trialeurodes vaporariorum* in Relation to "Lights On"

During development of the culture method described above, it was noticed that the majority of adult *T. vaporariorum* seemed to emerge a few hours after "lights on". Since investigation of the visual behaviour of *T. vaporariorum* required insects of a known physiological age, this phenomenon, if real, had potential usefulness and was investigated further.

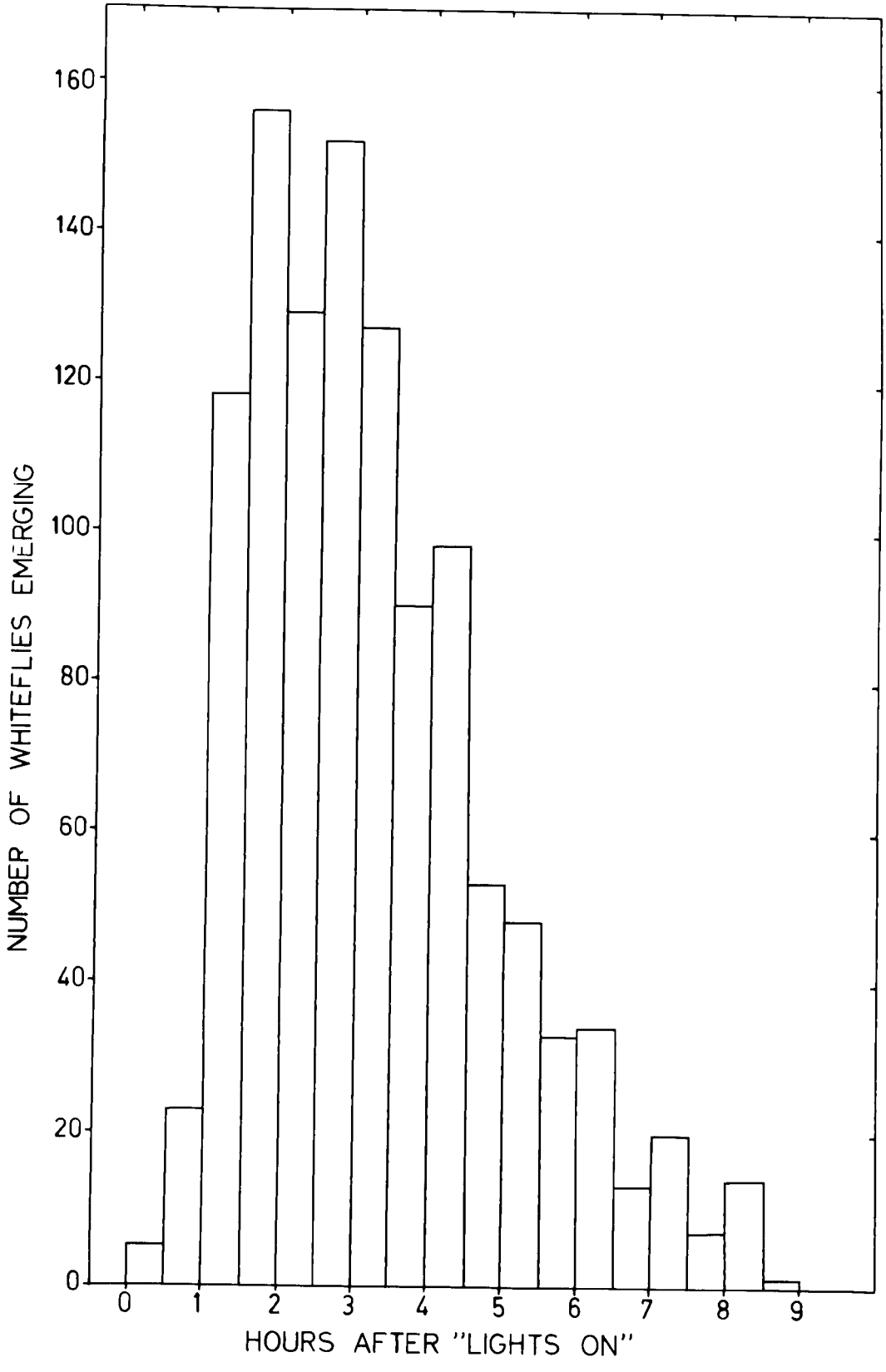
Two bean plants with mature fourth instar larvae on the first two leaves, were selected from the culture and washed down with a jet of water in the afternoon. These plants were placed in a growth cabinet at 21^{o}C. Immediately the lights came on in the morning the few adults which had emerged overnight were removed. Every half-hour, adults were collected with an aspirator as they emerged, and were counted.

Figure 2.1 shows that most adults emerged one to four hours after the lights came on, with the peak emergence occurring after 1.5-3 hours. These results do not necessarily mean that the adults were emerging in response to "lights on". The time the light came on relative to solar time was not varied, and it is possible that the whiteflies could have been responding to some other completely unrelated factor such as the time the lights went out the previous day. However, the aim of the experiment was to determine when the adults emerged so that a method for collecting experimental batches of whiteflies of a known age could be developed. The factors that may have stimulated emergence were not important since they remained constant.

2.3 Collection of Experimental Batches of Insects

Whiteflies to be used for experimental purposes were collected between 3-4 hours after the lights came on, from plants which had been washed with a jet of water on the previous afternoon. This means that, referring to Figure 2.1, the age difference within a sample was a maximum of 4.5 hours, but the vast majority would have had an age difference of 0-3 hours. Usually insects were collected with an aspirator connected to an aquarium pump, but when large numbers were needed they were gently shaken onto young plants and any that were not dislodged collected with the aspirator. Any adults that had emerged overnight were easily recognised by their waxy white wings which contrasted with the almost transparent wings of newly emerged whiteflies. Only whiteflies with transparent wings were collected. Once these insects had been collected, they were kept on bean plants at 21^{o}C ($\pm$ 1^{o}) in a growth cabinet until needed for experimental purposes.

Figure 2.1 Frequency distribution of whiteflies emerging each half hour interval from when the lights came on.


NUMBER OF WHITEFLIES EMERGING
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
HOURS AFTER "LIGHTS ON"

2.4 The Influence of Age on the Phototactic Response of *Trialeurodes vaporariorum* to white light

2.4.1 Introduction

Some preliminary observations on the phototactic behaviour of *T. vaporariorum* seemed to indicate that the response changed with age. The effect of age on the photactic response of *T. vaporariorum* to light was tested. White light alone was used because there was insufficient time to test the spectral characteristics of the response with age. Observations of whiteflies in a clean plexiglass cylinder of the same dimensions as the three-section apparatus described below indicated that most of the movement of undisturbed whiteflies toward monochromatic light was by walking. The response measured by the three-section apparatus with monochromatic light was therefore "slow" phototaxis, analogous to slow phototaxis in *Drosophila melanogaster* (Jacob *et al.*, 1977; Heisenberg and Götz, 1975; Fischbach, 1979). The response to white light in the plexiglass tube could not be termed "slow" phototaxis, however, because the whiteflies were much more active and there was a considerable number of short flights, compared with their behaviour under the intensities of monochromatic lights used.

2.4.2 Methods

(a) General - The three-section apparatus and its method of use

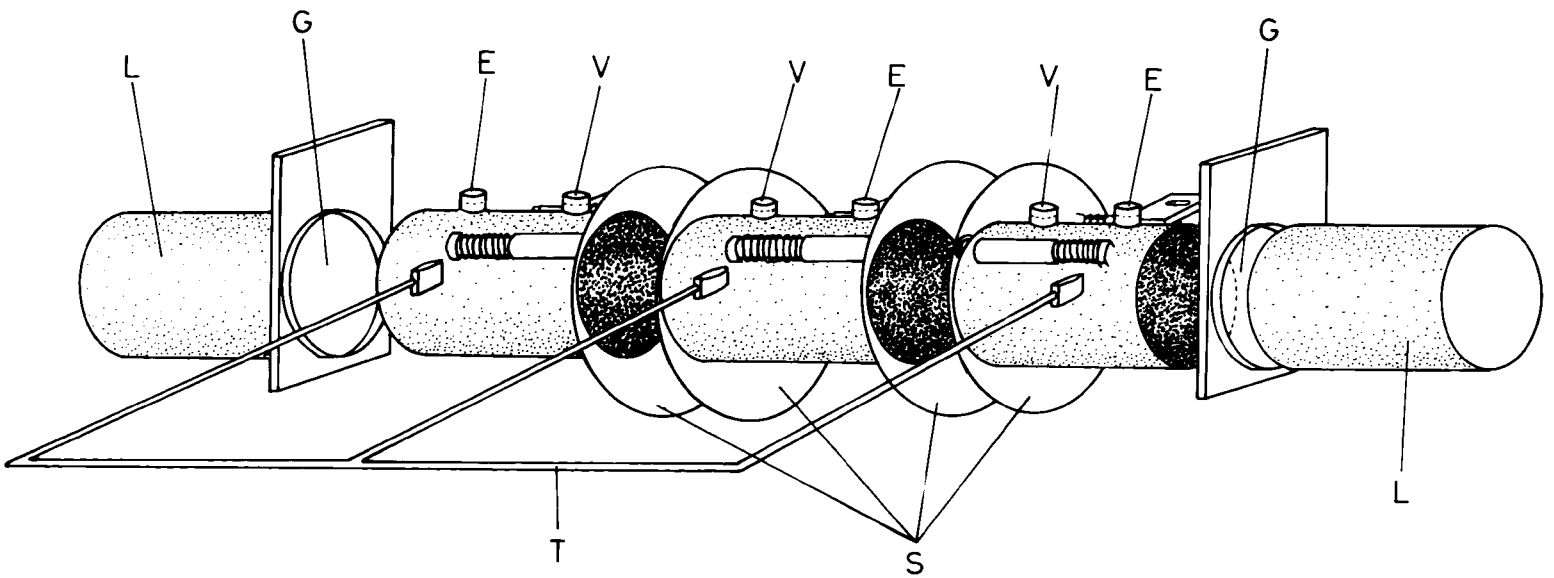
A cylindrical cage was constructed 150mm long and 26mm diameter, which could be divided instantaneously into three sections by

spring-loaded rotating shutters (see Figure 2.2). This apparatus was constructed of metal painted a matt black inside and out with moving parts dry-lubricated with graphite. Each section consisted of a tube 5cm long with one spring-loaded rotating shutter (two in the case of the middle section), one air vent with a sealing cap, and a hole by which the insects were introduced, also with a sealing cap. The ends of the cage consisted of ultra-violet-transmitting glass (Corning 9741). It was constructed in such a way that the whole apparatus could be dismantled readily and insects shaken out to be counted. While dismantled, the apparatus was cleaned with distilled water before each trial in order to remove any odours which may have been left by the previous batch of insects. A light-proof box was also constructed with two opposite holes to fit the ends of the cage so that a light could be shone through the length of the cage when mounted in the box. This apparatus utilises the same principle used by McEwen (1918), Dürrwächter (1957), Pak *et al.* (1969) and Fischbach (1979), i.e. measurement of the position of an insect or group of insects along a beam of light, where the light direction is parallel to the direction of movement of the animal.

Light flux was measured 7mm from the U.V. transmitting glass with a Reeder thermopile connected to a Keithley 249 milli-microvoltmeter. It was not possible to measure the light intensity inside the three section apparatus because of the size of the thermopile detector, but by mounting the detector in the light-proof box in the position normally taken by the three-section apparatus, a reasonable approximation was obtained. The three-section cage was

Figure 2.2 Expanded diagram of the three-section apparatus. G, ultraviolet transmitting glass (Corning 9741); E, entrance hole through which the whiteflies were introduced; V, air vent; L, light shield; S, spring loaded rotating shutters; T, shutter trigger.

The whole apparatus was mounted in a light-proof box.


L
G
E
V
V
E
V
E
G
L
T
S

mounted in the light-proof box with the shutters open and cocked, and insects were introduced with an aspirator through the holes constructed for this purpose. Equal numbers (30 per section) were put into each section in random order. The whiteflies were then dark-adapted for fifteen minutes. Fifteen minutes was based on the time required for dark adaptation in the bee and *Provespa nocturna* as determined by electrophysiological experiments (Burkhardt and de la Motte, 1972). After dark adaptation, the whiteflies were exposed to the light by removing a light-proof end cap and moving a shield aside which had been placed in front of the light source during dark adaption. The end through which the light was shone was alternated from replicate to replicate. After a set time had elapsed, the shutters were closed and the insects anaesthetised with CO_2. The cage was then dismantled, the whiteflies shaken out, and the number in each section counted. All these insects were discarded and the cage cleaned with distilled water. No insect was used more than once.

All the experiments described above were carried out in a darkroom at 25°C ($\pm$ 1°) with relative humidity normally 40-50% but occasionally up to 60%.

(b) Experimental

Newly emerged whiteflies were collected and placed on young bean plants in a growth cabinet at 21°C ($\pm$ 1°). Zero - 3 hours, 5-8 hours, 1 day, 1 week and 2 weeks from emergence, a sample of 90 insects was taken and their phototactic response to white light measured as described above. The light source was a 150W high pressure Xenon arc lamp (Bausch & Lomb) with a long wave infra-red filter

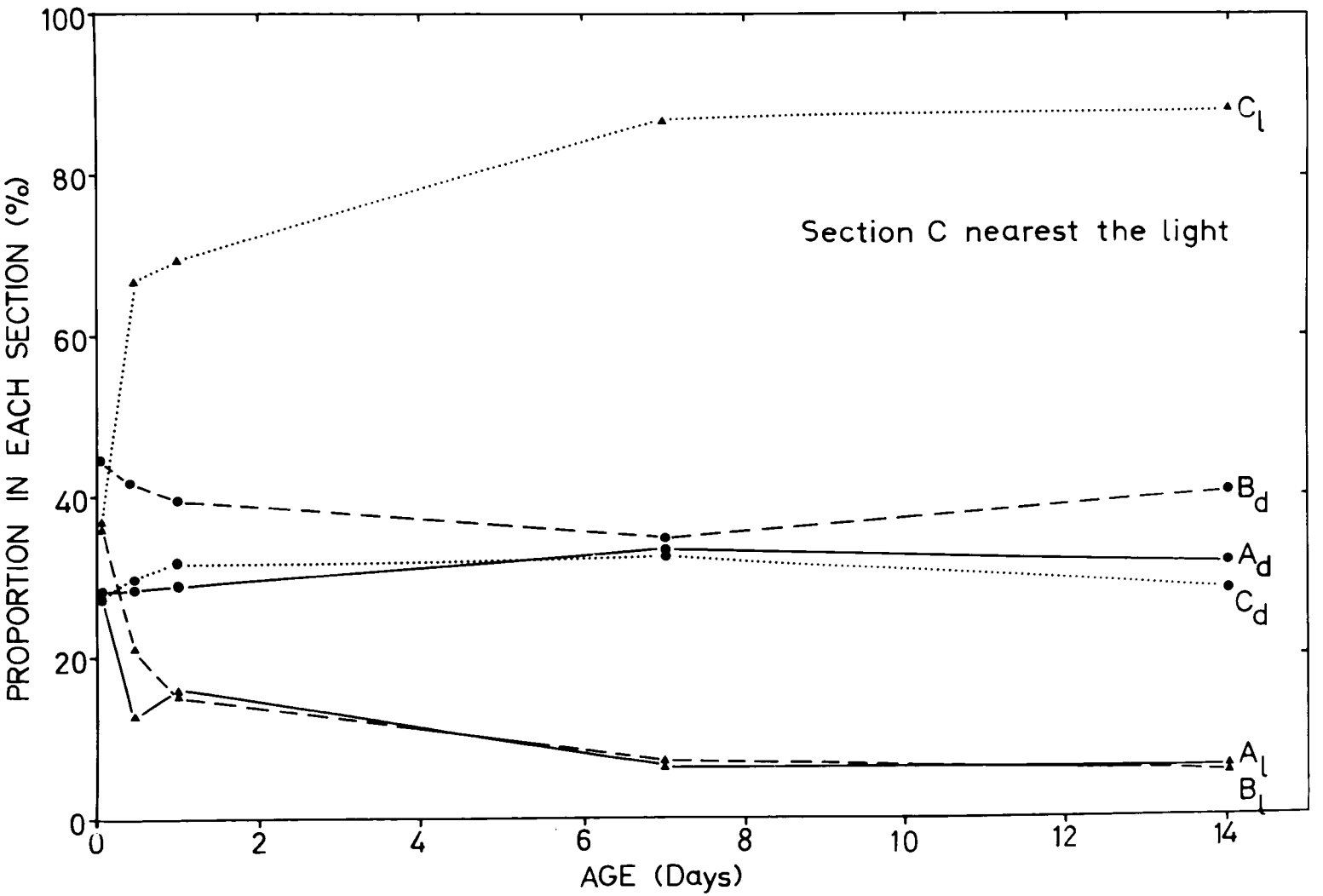
consisting of a quartz cell of path length 1cm filled with distilled water. The light flux density was 1,000 μW cm^{-2}. This level was arbitrary. Although phototactic response can change in magnitude or sign with intensity of white light in many insects (Jander, 1963) and crustacea (Pardi and Papi, 1961), it was impractical to vary light intensity as well as physiological age, because the information that would have been gained did not warrant the extra time. Exposure to light was for 2 mins. The experiment was replicated ten times and the data converted to percentages to remove errors in the total number of insects used in each trial due to escape or counting errors. The null hypothesis that there was no difference in the proportions in each section was tested with the Kruskal Wallis H test.

2.4.3 Results and Discussion

Mean proportions in each section with age are plotted in Figure 2.3. There is a significant difference at the 0.05 level between the proportions in each section for the control (i.e. in the dark) at 0.3 hrs, 3-8 hrs, 1 day and 2 weeks old and in all cases when the light was on. The difference in the dark indicates that the apparatus is biased towards the centre section, but after 5-8 hrs this bias is negligible and is unlikely to influence the results when the light is on, because the phototactic response is relatively large.

These results showed that *T. vaporariorum* is strongly photopositive and that the response develops rapidly with age. On this basis, one-day old adults were used in all further experiments.

Figure 2.3 Proportion of whiteflies in Sections A, B and C of the three-section apparatus plotted against age. Section C is nearest the light; l, light on; d, darkness (control). All points are the mean of ten replicates.


100
80
60
40
20
0
PROPORTION IN EACH SECTION (%)
0
2
4
6
8
10
12
14
AGE (Days)
Section C nearest the light
C_l
B_d
A_d
C_d
A_l
B_l

CHAPTER 3

3. SPECTRAL EFFICIENCY FUNCTION OF THE "SLOW" PHOTOTACTIC RESPONSE

3.1 Introduction

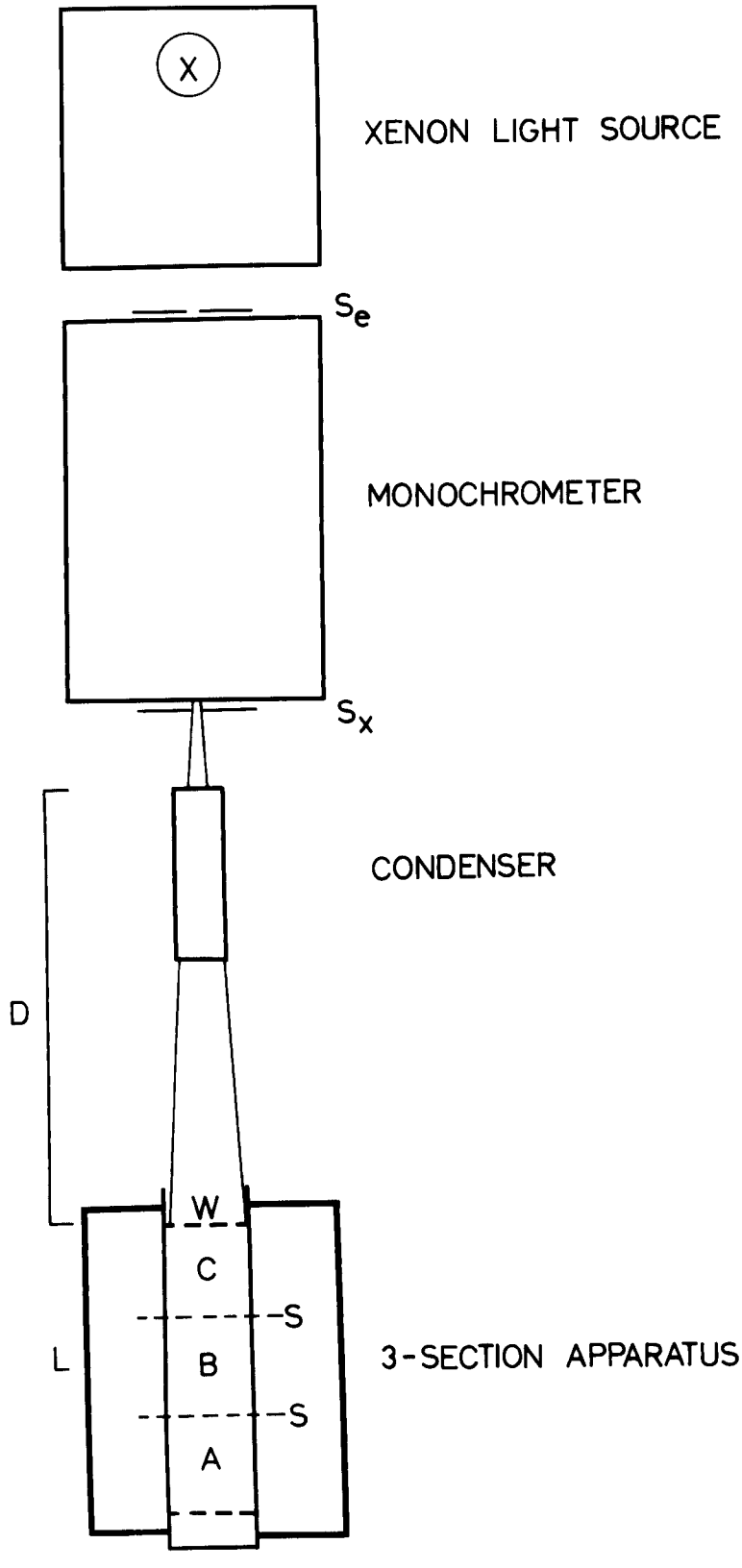
Lloyd (1922) first reported a "colour tropism" of *Trialeurodes vaporariorum* when he found whiteflies were trapped in greater numbers on sticky yellow traps than on other colours. Moericke *et al.* (1966) found that cessation of wing movement and "fall reflex" was stimulated by a yellow card when it was placed below a whitefly that was tethered by the prothorax to a needle and was flapping its wings as if in flight. This response did not occur when the yellow card was placed above or in front of the whitefly and it did not occur if the card was any of a series of twenty grey colours, ranging from white to black. MacDowall (1972) determined a "phototactic action spectrum" for whitefly and investigated colour vision using a colorimetric method. MacDowall's "action spectrum", which is really a spectral efficiency function, shows a peak at 550nm and closely follows the transmission spectrum of a tobacco leaf. However, this function only covered the range of wavelengths from 475 to 625nm. He found no evidence for colour vision. Vaishampayan *et al.* (1975a) measured the response of *T. vaporariorum* to reflected and transmitted light of various colours and found that most whiteflies were trapped on a yellow surface. Smaller numbers were trapped on an unsaturated yellow surface than a saturated yellow, even though there was more light reflected from the unsaturated surface across the spectrum (400-700nm). Light reactions of other species of whiteflies have been investigated by Butler (1938), Husain and Trehan (1940) and Mound (1962). However, these results are difficult to

interpret because the role of ultra-violet light has been neglected and the intensity has not been sufficiently controlled, except perhaps in MacDowall's (1972) experiments. Because of this, and bearing in mind the conclusions reached in a previous study (Coombe, 1976) the spectral efficiency was investigated further.

3.2 Methods

One-day-old whiteflies were collected as described in Section 2.3. The "slow" phototactic response to various wavelengths of light of equal quantum flux was measured in the apparatus described in Section 2.4.2(a). The response measured by this apparatus illuminated by monochromatic light is "slow" phototaxis because the whiteflies are undisturbed, and most of the movement towards the light is by walking (see Section 2.4.1). It was impractical because of insufficient time to vary the quantum flux level as well as wavelength. The light source was a Bausch and Lomb high intensity grating monochromator with a UV-visible grating, Xenon light source and quartz-fluoride condenser. The monochromator slit widths were adjusted to give a one-half band-width of 10nm. Appropriate cut off filters (Kodak Wratten filters Nos. 2B, 4, 16 and 25) were used to absorb higher order wavelengths. The light intensity was varied by moving the condenser and the light-proof box, which remained 63cm from the condenser, towards or away from the monochromator. The condenser was kept focussed on the front of the light-proof box. By this method, the light intensity could be varied without changing the light gradient (see Figure 3.1). The wavelengths used were 350, 400, 450, 500, 550 and 650nm with a quantum flux of 9.07×10^{13} quanta $cm^{-2} sec^{-1}$. The insects were exposed to the light for three minutes, after which the shutters were closed. The experiment

Figure 3.1 Schematic representation of the apparatus used to measure "slow" phototaxis. X, Xenon light source; S_e, entrance slit; S_x, exit slit; D, distance of 63cm; W, ultra-violet transmitting glass (Corning 9741) window; A, B, C, sections of the three-section apparatus; S, spring-loaded rotating shutters; L, light-proof box.


X
XENON LIGHT SOURCE
S_e
MONOCHROMETER
S_x
CONDENSER
D
W
C
S
L
B
S
A
3-SECTION APPARATUS

was replicated ten times and the order at which each wavelength was tested was randomised within each replicate. The end of the apparatus through which the light was shone was alternated from replicate to replicate. Statistical analysis was by a split-plot design analysis of variance. Analysis of variance was used rather than a simple χ^2 because the data was found to be non-homogeneous in a number of preliminary experiments.

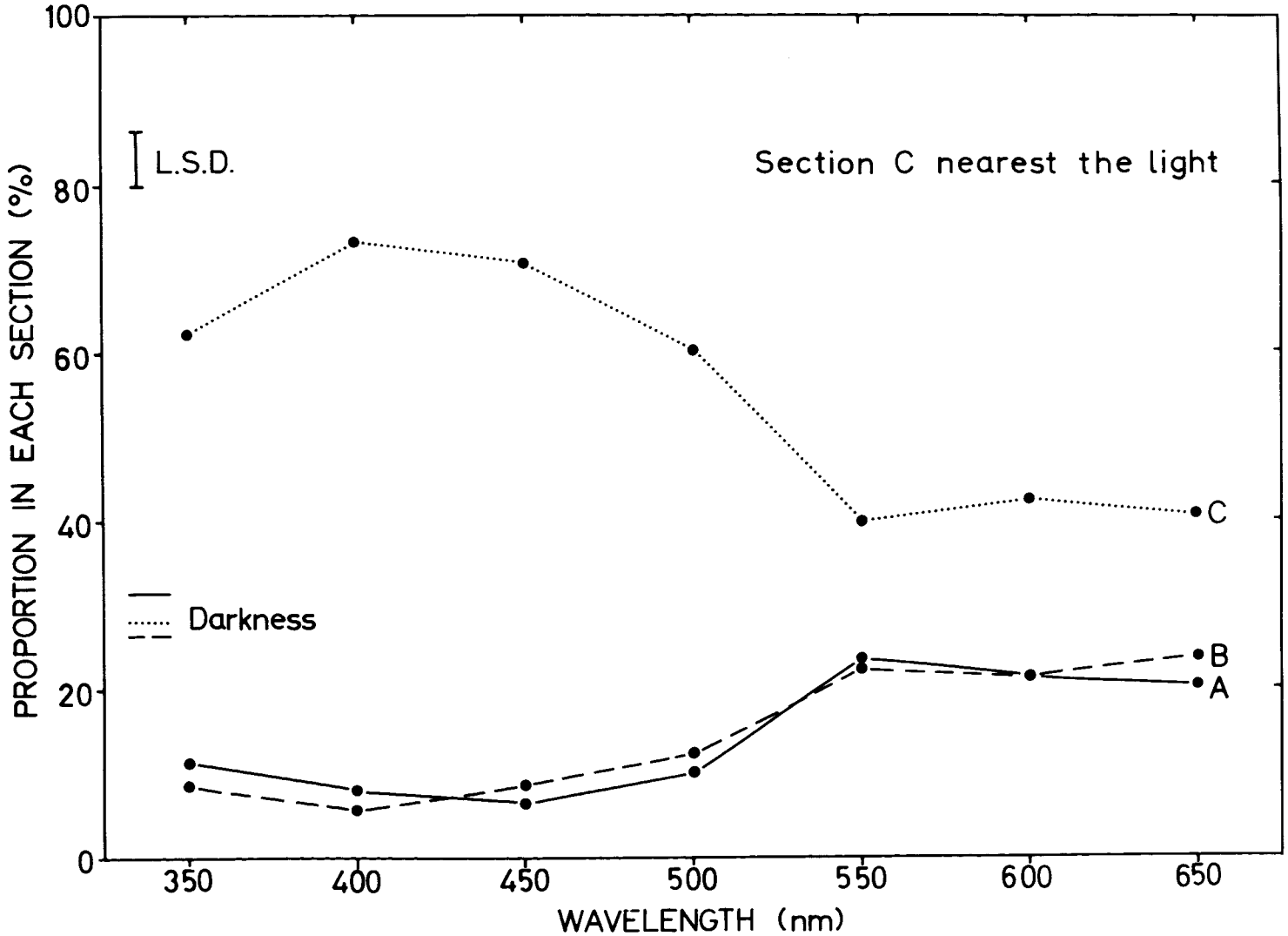
3.3 Results and Discussion

Mean numbers of whiteflies in each section are plotted against wavelength in Figure 3.2. Considering the numbers in Section C (closest to the light), since the numbers in this section probably represent the best measure of phototactic activity, there is a peak at 400-450nm and a relatively low phototactic activity at 550nm and longer wavelengths. This result contrasts with that of MacDowall (1972) who found a peak at 550nm. The difference between these two sets of results indicates that either, (1) the different design of the experiment has introduced a factor which had influenced the behaviour of the whiteflies, (2) a different behavioural pattern is being measured, or (3) the insects themselves are different. Factors which could have influenced the shape of the spectral efficiency function are:-

(1) Dark adaptation. In the experiment described above, the whiteflies were dark adapted for fifteen minutes before the response was measured, whereas MacDowall's whiteflies were probably light adapted.

(2) Quantum flux. MacDowall's quantum flux level was approximately six times greater. A smaller or greater light flux may have produced

Figure 3.2 Spectral efficiency function of "slow" phototaxis. Proportion of whiteflies in each section (%) plotted against wavelength of light. L.S.D., least significant difference (5% level). Each point is the mean to ten replicates.


100
80
60
40
20
0
PROPORTION IN EACH SECTION (%)
L.S.D.
Section C nearest the light
Darkness
C
B
A
350
400
450
500
550
600
650
WAVELENGTH (nm)

a spectral efficiency function of a different shape because univariance does not necessarily hold for this behavioural pattern (Menzel, 1979).

(3) Physiological age. The physiological age of the whiteflies used in the experiment described above was one day old at 21°C whereas the age of MacDowall's whiteflies almost certainly varied. Age has been shown to influence phototaxic responses, e.g. *Drosophila melanogaster* (McEwen, 1918; Hadler, 1964; Dürrwächter, 1957).

(4) Temperature and/or relative humidity. Temperature has been shown to affect the magnitude or sign of phototaxis, e.g. *Calliphora erythrocephala* (Jander, 1963), *Glossina morsitans* (Jack and Williams, 1937), *Chlenias pachymela* (Khoo, 1979), *Blastophagus piniperda* (Perttunen, 1958). Relative humidity and desiccation can also affect phototaxis (Hadler, 1964; Perttunen, 1963; Perttunen and Lahernaa, 1958). The above experiment was carried out at 25°C±1° and a relative humidity of mostly 40-50% but occasionally up to 60%. MacDowall does not state the temperature or the relative humidity at which his experiments were carried out.

(5) Other factors such as length of light exposure, agitation, handling of the insects, density or rearing techniques may have had an effect.

In order to determine which of the above factors may have influenced the response observed, some observations of whitefly behaviour under varying conditions were made in a clear plexiglass tube of the same dimensions as the three-section apparatus. Illumination was switched alternatively from 400nm to 550nm (9.07 quanta $cm^{-2}sec^{-1}$) and any behavioural changes were noted. Although no quantitative data

were obtained due to insufficient time, there was a marked difference in behaviour at the two wavelengths, i.e. the whiteflies walked faster towards the 400nm light. This difference was used to give some indication of what factors may have influenced the "slow" phototaxis response. The factors investigated were:-

(1) Temperature - 15°C, 20°C, 25°C and 30°C.

(2) Light intensity - increased approximately four times by increasing the bandwidth of the monochrometer to 20nm.

(3) Light *vs* dark adaptation.

(4) Age - one week old *vs* one day old.

(5) Density - 50, 90 and 200 insects per trial.

(6) Disturbance - the tube was shaken immediately before the light was turned on.

Only a small number of combinations of the various factors could be investigated but in all cases tested (usually standard conditions except for one factor), the whiteflies walked faster towards the 400nm light. From these results it was concluded that none of the above factors were likely to have influenced the spectral characteristics of the "slow" phototactic response. However, movement towards the monochromatic light was mostly by walking and therefore there is a possibility that the behavioural response measured was different from MacDowall (1972). In the experiment described above, the number of insects walking along a light gradient was measured, whereas MacDowall

measured the number on a window 10-15 sec. after agitating the container and turning the light on. Thus, it is likely that flight, landing and take-off were involved rather than walking, i.e. MacDowall almost certainly did not measure "slow" phototaxis. Different behavioural patterns may have different intensity and/or spectral characteristics, e.g. *Drosophila pseudoobscura* (Lewontin, 1959), *D. melanogaster* (Hu and Stark, 1977), *Apis mellifera* (Labhart, 1974; Kaiser *et al.*, 1977; Edrich, 1979; Menzel, 1979) and the apparatus can influence the response observed, either because different behavioural patterns are being measured or because of differences in the magnitude of the intensity gradient in the test apparatus (Fischbach, 1979), e.g. Jacob *et al.* (1977) c.f. Fischbach (1979). The state of light or dark adaptation level can determine which visual mechanisms dominate phototaxis (Schümperli, 1973; Heisenberg and Götz, 1975; Hu and Stark, 1977). Thus, in order to explain the difference of the results obtained with the three-section apparatus from those of MacDowall (1972), and to gain a broader insight into the visual behaviour of *T. vaporariorum*, a number of different pieces of apparatus and several methods were used. In addition, the technique using the three-section apparatus was too tedious and the apparatus itself too unreliable to attempt any further investigations using this method. A simpler, less laborious and more reliable method was needed in order that further work on wavelength specific behaviour and colour vision could be attempted.

CHAPTER 4

4. ASSESSMENT OF DIFFERENT BEHAVIOURAL RESPONSES OF *TRIALEURODES VAPORARIORUM*

4.1 Introduction

The aims of the series of experiments described in this chapter were, (1) to gain a broad insight into the wavelength dependence of the visual behaviour of *Trialeurodes vaporariorum* and to relate this to food-plant selection, (2) to attempt to explain the difference between the results obtained in Chapter 3 (see Figure 3.1) and those of MacDowall (1972), and (3) to develop a technique that could be used for further investigations into wavelength specific behaviour and colour vision. In order to understand the settling and dispersive behaviour of whiteflies it was necessary to break the behaviour into various components, i.e. walking, take-off, flight and landing behaviour. This was achieved by the use of different methods and/or apparatus, coupled with an analysis of each method to determine what aspect of behaviour each method measured. Thus the hypothesis that the difference between my results (Figure 3.1) and those of MacDowall (1972) was due to the different behavioural patterns used (which had different spectral sensitivities), was tested. The technique used in Chapter 3 was too laborious and the apparatus too unreliable to attempt further investigations into colour vision and so an improved method was needed. The time and resources were not available to develop an automatic, labour-saving apparatus such as those described by Heisenberg and Götz (1975), Menne and Spatz (1977) and Fischbach (1979) for *Drosophila melanogaster*. Because of this, the methods developed were necessarily

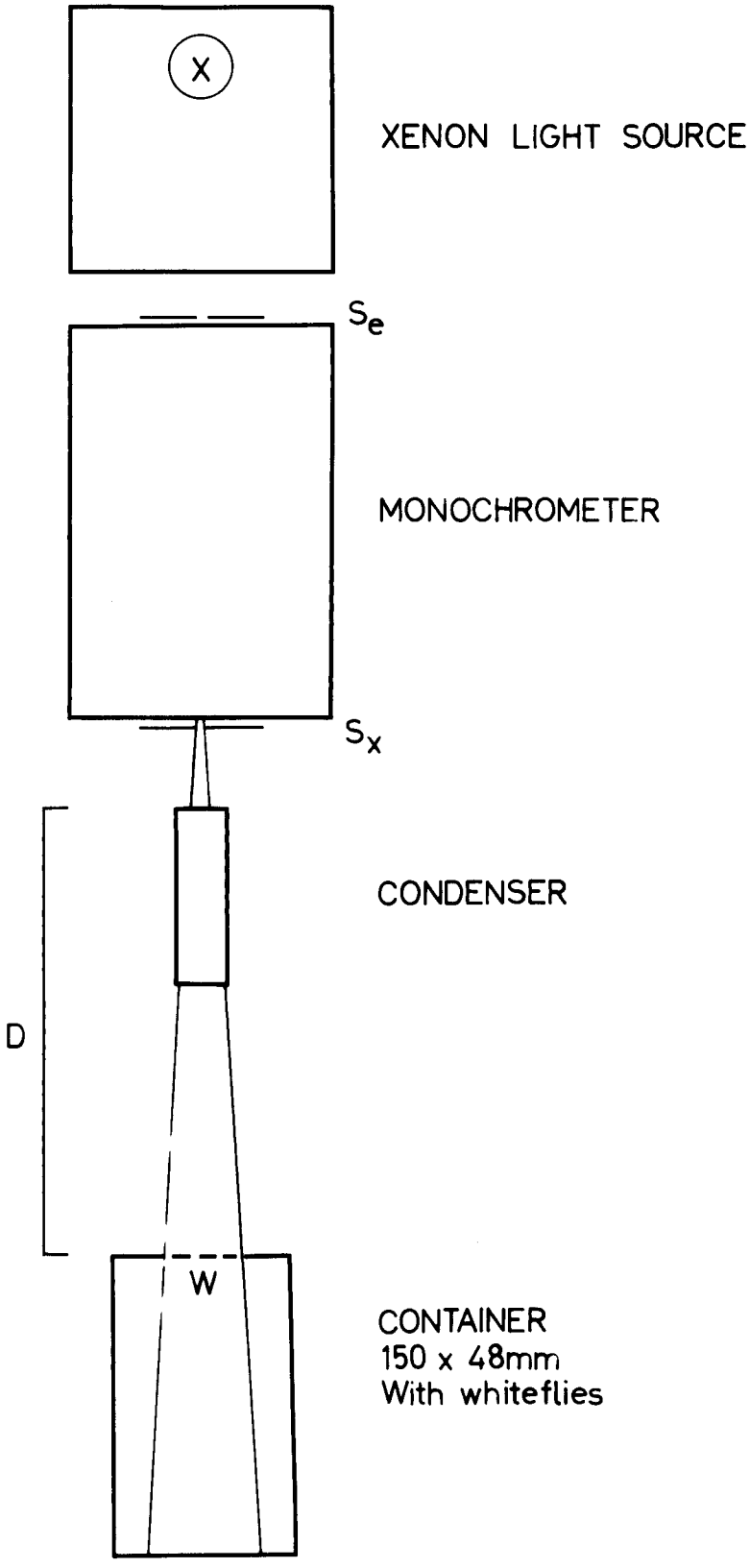
crude. Also it was important to keep in mind that *T. vaporariorum* adults are delicate and if removed from a plant will not live more than a few hours at 25°C and 50% relative humidity, i.e. they would die in the apparatus described by Heisenberg and Götz (1975). In addition, they are smaller than *Drosophila* and are very much less active under the same conditions. All the methods described in the following sections measured the response of the whiteflies over a short period of time with each individual insect being used once only (except for experiment 9). This minimised any change in response which may have occurred because of fatigue, learning or desiccation over the time course of the experiments.

4.2 Experiment 1. Duplication of MacDowall's (1972) Method

The aim of this experiment was to test whether the whiteflies used by MacDowall (1972) were similar, by duplicating as closely as possible the method used by MacDowall in his study on phototaxis in *T. vaporariorum*.

A cylindrical container (150mm long, inner diameter 48mm) was constructed of black plastic except for a clear plexiglass window 3cm in diameter at one end (see Figure 4.1). A monochromatic light was shone through this window, either 400nm or 550nm, with a quantum flux of 9.07×10^{13} quanta cm^{-2} sec^{-1}, which corresponds to the intensity used in Chapter 3. These wavelengths were chosen because 400nm corresponds to the peak in the spectral efficiency function of the "slow" phototactic response and 550nm corresponds to the minimum (see Figure 3.1). 550nm also corresponds to the peak of MacDowall's spectral efficiency function. The light source and angle of the light beam, adjustment of light intensity and the method of measurement of intensity

Figure 4.1. Apparatus used to duplicate MacDowall's method of measuring phototaxis in *T. vaporariorum*. S_e, entrance slit; S_x, exit slit; D, distance of 63cm; W, clear plexiglass window.


X
XENON LIGHT SOURCE
S_e
MONOCHROMETER
S_x
CONDENSER
D
W
CONTAINER
150 x 48mm
With whiteflies

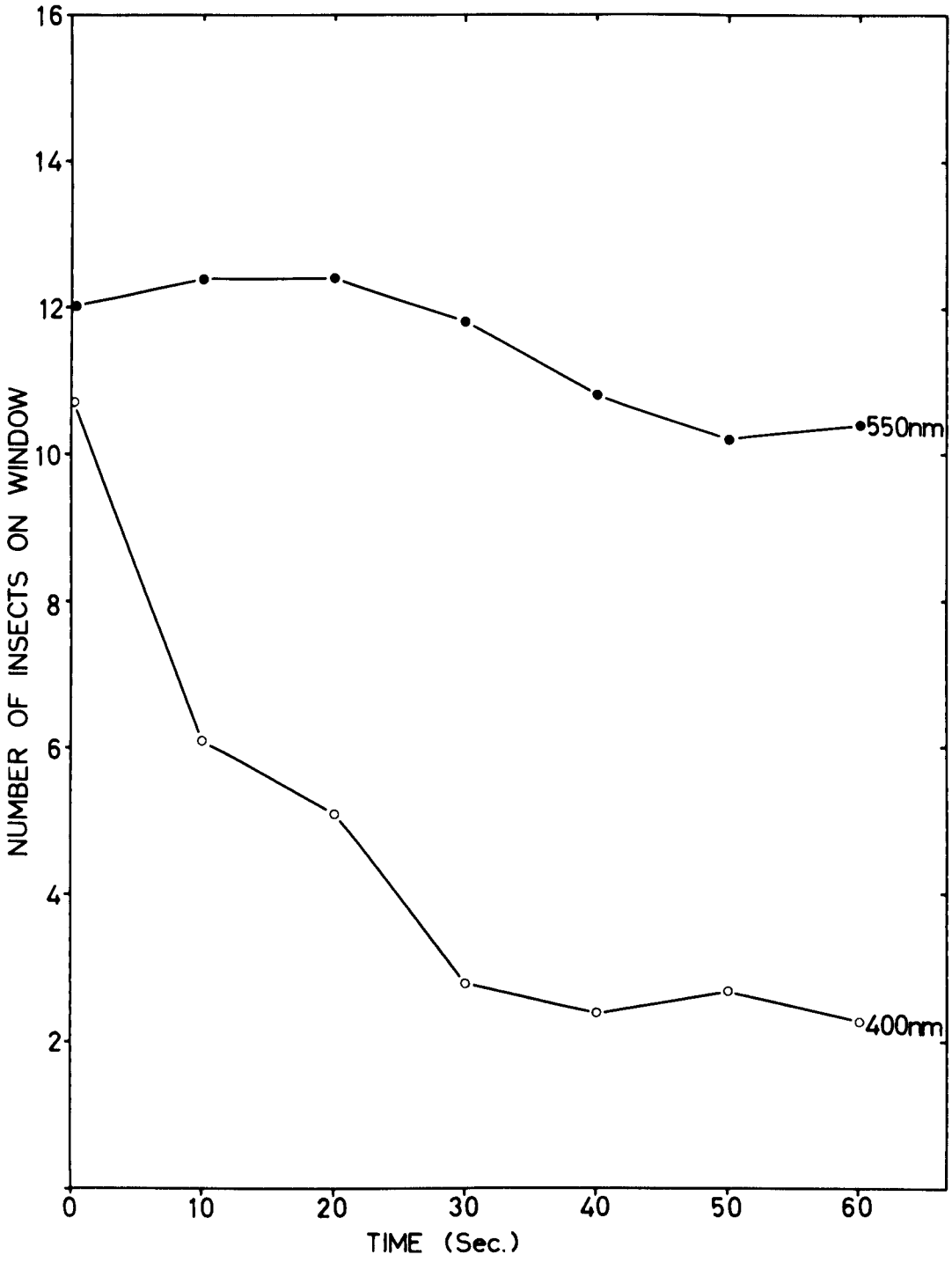
was identical to that used for the "slow" phototactic response (Chapter 3).

Approximately four hundred one-day-old whiteflies were placed in the container and dark adapted for fifteen minutes. The container was then shaken, the light turned on immediately after shaking, and the number of insects on the window counted at ten second intervals for one minute. This was repeated ten times at each wavelength, with the order in which the lights were presented randomised in order to minimise effects of increasing age during the day. Statistical analysis was by analysis of variance with a square root transformation.

The results are summarised in Figure 4.2. The mean number of insects on the window illuminated with 550nm light did not differ significantly over the time interval, but there was a significant decrease in the mean number on the window illuminated with 400nm. These results conflicted with those obtained using the three-section apparatus described in Chapter 3 (in which the animals showed "slow" phototactic behaviour), but agreed with MacDowall's (1972) results. The reason for the difference between the results of the experiment testing the "slow" phototactic response and the results of the above experiment could have been due to the differing designs of the containers (different overall size and different relative size of the window) and/or the different experimental methods used (numbers on the window compared with the number in each section) could have measured different behavioural responses.

In this experiment, a deliberate attempt had been made to duplicate MacDowall's method as closely as possible. In particular the inner surface of the container used in the above experiment was not

Figure 4.2 Duplication of MacDowall's method. The number of insects on the window is plotted against the time from when the light was turned on. Each point is the mean of ten replicates.


16
14
12
10
8
6
4
2
NUMBER OF INSECTS ON WINDOW
550nm
400nm
0
10
20
30
40
50
60
TIME (Sec.)

in the beam of light (see Figure 4.1), hence for a whitefly to intercept the light beam and respond to it, that whitefly would have to be in flight, whereas the experiment using the "slow" phototactic response (Chapter 3) recorded the behaviour of insects that were walking. The relatively low proportion of test insects observed on the window in all such experiments may have been due to a low number of insects in flight at any one time, and what was measured in the above experiment was probably a combination of flight, landing and take-off behaviour; this combination could well have had different spectral characteristics from "slow" phototaxis. However, before any definite conclusion could be made, the influence of the design of the container and the method of measurement of the response needed to be investigated.

4.3 Experiment 2. The Influence of Window Size and Method of Measurement

The aim of this experiment was to determine the effect of, (1) the relative size of the window (which probably influences the behavioural pattern being measured), and (2) the effect of the method used to measure the phototactic response.

To eliminate any size factor, the original three-section apparatus was used. "Slow" phototaxis was measured as described in Chapter 3 by counting the number in each section after three minutes (Method 1). The number on the window (corrected for differing sizes of window) at various time intervals after the light was turned on, was measured by photographing the window using the monochromatic light

as a light source (Method 2). Two sizes of window were used - 3cm, i.e. the full diameter of the container, and 1.5cm. The wavelengths used were 400nm and 550nm. Thus the treatments were -

(1) Number in each section (Method 1), small window 550nm.

(2) Number in each section (Method 1), small window 400nm.

(3) Number in each section (Method 1), large window 550nm.

(4) Number in each section (Method 1), large window 400nm.

(5) Number on the window (Method 2), small window 550nm.

(6) Number on the window (Method 2), small window 400nm.

(7) Number on the window (Method 2), large window 550nm.

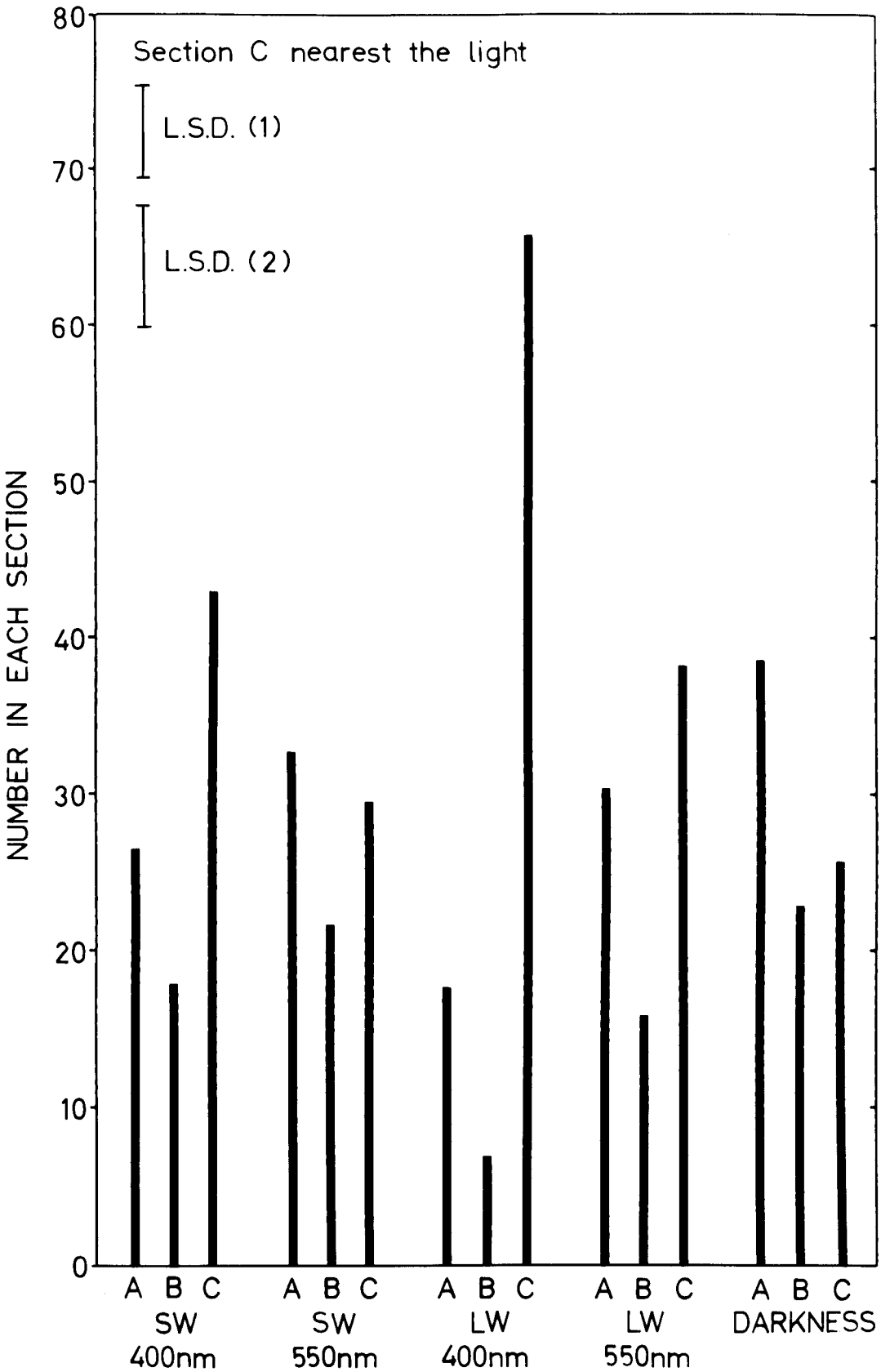
(8) Number on the window (Method 2), large window 400nm.

(9) Number in each section (Method 1), darkness.

The light source, measurement and adjustment of light intensity and the intensity were identical to that used in Chapter 3. Statistical analysis was by a split-plot design analysis of variance for Method 1 and a Kruskal-Wallis H test for Method 2. A non-parametric test was used for data obtained with Method 2 because normality could not be assumed since the numbers recorded were low.

Method 1 - From Figure 4.3 it can be seen that for both window sizes the response to 400nm is greater than to 550nm and the response with the large window is greater than for the small window for both wavelengths.

Figure 4.3 Influence of window size and method of measurement. Method 1. Number of whiteflies in each section of the three-section apparatus plotted against the various treatments. A, B, C, various sections of the apparatus, with C being closest to the light; SW, small window; LW, large window; L.S.D. (1) least significant difference (5%) between treatments; L.S.D., least significant difference (5%) between sections within treatments. Each plotted value is the mean of ten replicates.


Section C nearest the light
L.S.D. (1)
L.S.D. (2)
NUMBER IN EACH SECTION
80
70
60
50
40
30
20
10
0
A B C
SW
400nm
A B C
SW
550nm
A B C
LW
400nm
A B C
LW
550nm
A B C
DARKNESS

Method 2 - From Figure 4.4 it can be seen that the mean number on the large window increased with time, with significantly more insects recorded when the container was illuminated with 400nm. The mean number on the small window, however, showed little change from what would be expected if the distribution of insects was random over the inner surface of the container. One would expect that the curve for the small window at 400nm would fall as was observed in the previous experiment (see Figure 4.2). However, the initial number on the small window was very low and the method was thus relatively insensitive.

It can be concluded from these results that the size of the window relative to the size of the container influenced the response observed, but the methods of measurement gave similar results. This supports the hypothesis put forward in the previous section that, because of the differing design of the containers, different behavioural responses were probably being assayed. In experiment 1 (Section 4.2) only whiteflies in flight would be able to respond to the light, whereas with "slow" phototaxis (Chapter 3) most of the movement towards the light was by walking. To further distinguish between these aspects of behaviour, methods were developed that broke the behaviour into its various components, i.e. walking, take-off, flight and landing behaviour.

4.4 Experiment 3. Walking and Take-off Behaviour of Individual Whiteflies

In this experiment the rate of walking and the frequency of take-off of individual *Trialeurodes vaporariorum* adults were measured at two wavelengths.

Figure 4.4 Influence of window size and method of measurement. Method 2. Number of whiteflies on the window plotted against time from when the lights came on. L400, large window, 400nm; L550, large window 550nm; S550, small window 550nm; S400 small window 400nm.

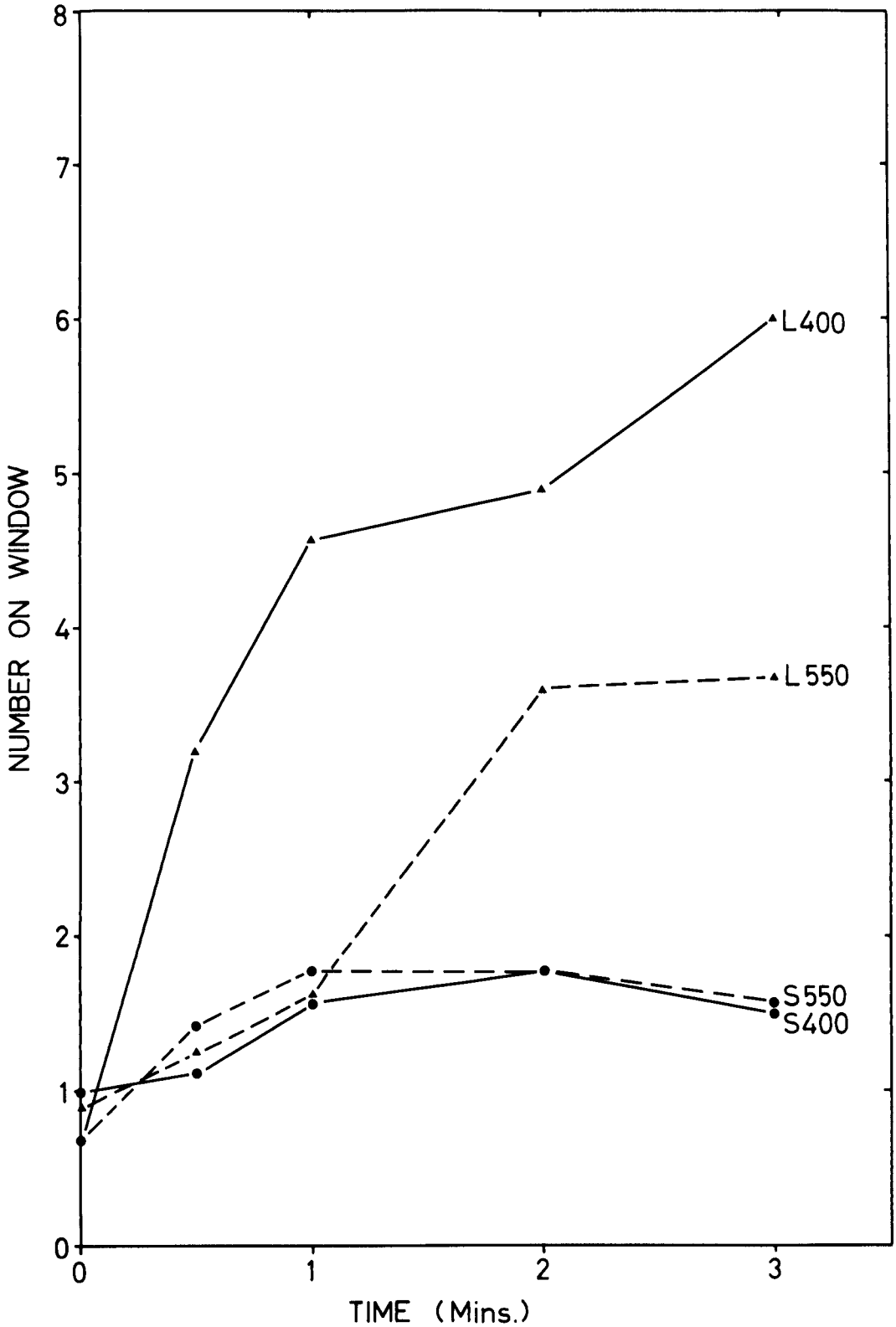

8
7
6
5
4
3
2
1
0
NUMBER ON WINDOW
0
1
2
3
TIME (Mins.)
L400
L550
S550
S400

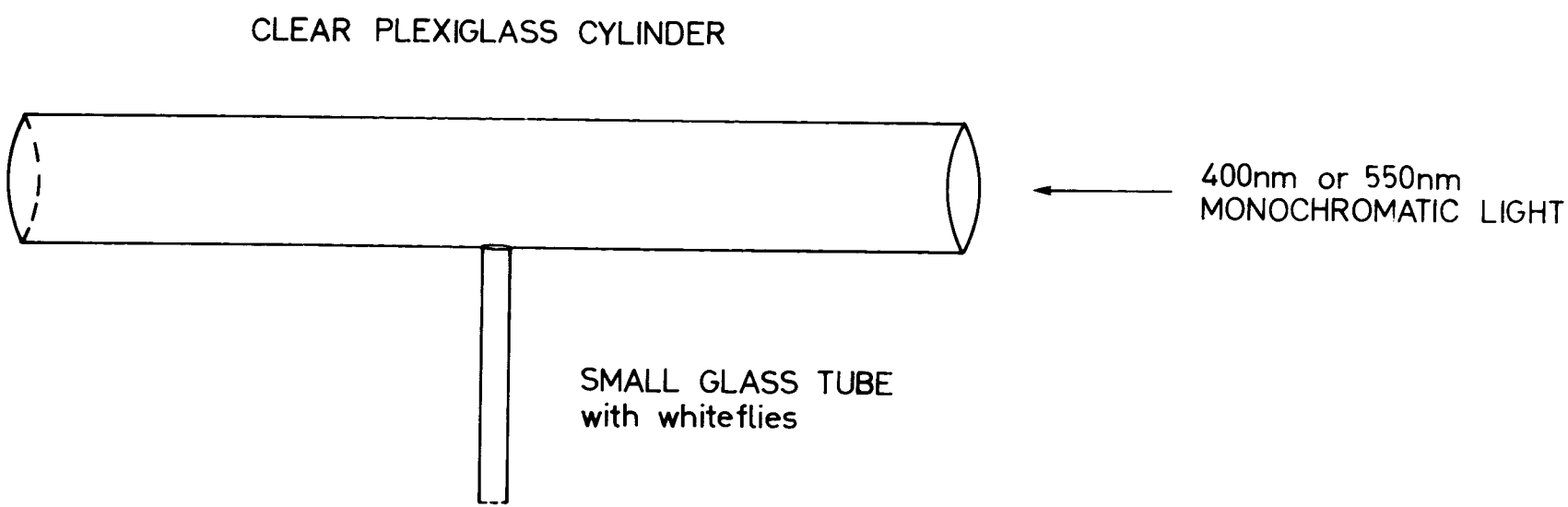
Table 4.1 Walking and Take-off Behaviour of Individual Whiteflies (Experiment 3).

	400nm	550nm	
Mean rate of walking (cm/min)	14.1	3.8	$p < 0.05$
Number of take-offs	15	6	$\chi^2_1 = 5.25$
Number of no take-offs	10	19	$p < 0.05$

A clear plexiglass cylindrical container was constructed 300mm long, inner diameter 25mm, open at both ends with a small hole 15cm from either end (see Figure 4.5). One-day-old whiteflies were dark adapted for at least fifteen minutes, in a small glass tube in a light-proof box, and introduced into the container by allowing them to walk up the glass tube and through the central hole (Figure 4.5). The distance walked towards the light in one minute, was recorded for all the insects that did not take off in this time interval. If the insect took off, the flight, the time of take-off and the distance walked before take-off were recorded. From these data, the rate of walking could be calculated. Since the error increases as the time spent walking and the distance walked decreases, the rate of walking was calculated only for those insects that walked for longer than ten seconds. The direction walked was recorded as a positive value if it was towards the light and negative if away from the light. A total of fifty insects was observed, 25 at each wavelength. The light source, method of measurement and adjustment of intensity, and the intensity were identical with those detailed in Chapter 3; thus light flux at the central hole was 9.06×10^{13} quanta $cm^{-2}sec^{-1}$. Two wavelengths were used, 400nm and 550nm, and were tested in random order. Statistical analysis was by a Mann-Whitney U test for the walking data and a 2x2 contingency table for the take-off data.

The results are summarised in Table 4.1. The net speed of walking was significantly greater toward the 400nm light than the 550nm light and the frequency of take-off was significantly greater in 400nm light than in 550nm, i.e. the whiteflies were more active under 400nm illumination than 550nm.

Figure 4.5 Diagram of the container used to observe walking and take-off behaviour of individual whiteflies. Whiteflies were collected in the small glass tube and allowed to walk up the tube into the cylinder.


CLEAR PLEXIGLASS CYLINDER
400nm or 550nm
MONOCHROMATIC LIGHT
SMALL GLASS TUBE
with whiteflies

Although the whiteflies were more active under 400nm illumination in respect to walking and take-off behaviour, the above experiment did not provide information on flight or landing behaviour. Since flight and landing behaviours are important components of the settling and dispersive behaviour of *T. vaporariorum*, and were components of the response measured in Experiment 1 (duplication of MacDowall's method), the experiment outlined below was designed to test the effect of the two wavelengths used (400nm and 550nm) on the orientation of *T. vaporariorum* while in flight. Landing behaviour was examined in later experiments.

4.5 Experiment 4. Flight Orientation

The aim of this experiment was to determine the wavelength of monochromatic light to which *T. vaporariorum* will orient when in flight, using the two wavelengths used in the two previous experiments. For this purpose, two monochromatic lights were presented simultaneously from opposite directions to an animal about to take off. The wavelengths were 400nm and 550nm, both had equal quantal fluxes which varied from 9.07×10^{13} quanta $cm^{-2} sec^{-1}$ to 0.5, 0.25 and 0.125 of this level. The intensity was measured with the thermopile detector at the point of take-off and was adjusted with Kodak Wratten neutral density filters. Two light sources were used, one a Bausch & Lomb high intensity grating monochromator with a UV-visible grating and Xenon light source, the other a slide projector fitted with an interference filter and powered by two twelve-volt batteries. In both cases the one half bandwidth was 10nm. A Wratten filter No. 4 was used with the 550nm light to absorb higher order wavelengths.

Table 4.2 Flight Orientation.

Intensity ($\times 9.07 \times 10^{13}$ quanta $cm^{-2} sec^{-1}$)	Frequency of Flight Towards 400nm	550nm	
1.0	158	42	$\chi^2_1 = 67.3$ $p < 0.05$
0.5	144	56	$\chi^2_1 = 38.7$ $p < 0.05$
0.25	132	68	$\chi^2_1 = 20.5$ $p < 0.05$
0.125	128	72	$\chi^2_1 = 15.7$ $p < 0.05$

Effect of intensity (4x2 contingency table) $\chi^2 = 13.09$ $p < 0.05$

Twenty insects were tested at one time, after which the direction of the lights was altered by changing the filter on the projector and the wavelength setting on the monochromator. The positions of the projector and monochrometer were not changed because this was a very cumbersome procedure.

Adult whiteflies, one day old, were placed in a small glass tube and dark adapted for at least fifteen minutes in a light-proof box. This tube was then placed vertically in the two opposite beams of light. The whiteflies walked up the glass tube and usually took off within a few seconds after reaching the top. The direction of flight after take-off was noted. A total of two hundred insects were tested at each intensity level.

The results were analysed statistically as follows. A test for bias was firstly carried out by using 2x10 contingency tables. These were all non-significant, indicating no evidence of any bias towards one light source or side. The data from the random trials were then pooled for each intensity and the null hypothesis that there was no difference in the frequency of flights towards 400nm compared with 550nm tested with a simple χ^2 test. A 2x4 contingency table was used to test the effect of intensity.

The results are summarised in Table 4.2. At all intensities, significantly more whiteflies flew towards the 400nm light but the proportion flying towards 400nm decreased as the intensity decreased.

From the above experiment it can be concluded that the whiteflies flew towards the shorter wavelength. However, in Experiment 1

Table 4.3 Trapping on a Sticky Surface.

Intensity ($\times 9.07 \times 10^{13}$ quanta $cm^{-2} sec^{-1}$)	Total No. of Whiteflies Trapped 400nm	550nm	
1	581	190	$\chi^2_1 = 198.3$ $p < 0.05$
0.5	397	176	$\chi^2_1 = 85.2$ $p < 0.05$
0.25	285	166	$\chi^2_1 = 31.4$ $p < 0.05$
0.125	194	118	$\chi^2_1 = 18.5$ $p < 0.05$
- ∞	13	13	n.s.

Effect of intensity (4x2 contingency table) $\chi^2_1 = 28.5$ $p < 0.05$

Table 4.4 Photography.

Intensity ($\times 9.07 \times 10^{13}$ quanta $cm^{-2} sec^{-1}$)	Total No. of Whiteflies Photographed 400nm	550nm	
1	97	243	$\chi^2_1 = 62.7$ $p < 0.05$
0.5	48	227	$\chi^2_1 = 116.5$ $p < 0.05$
0.25	54	146	$\chi^2_1 = 42.3$ $p < 0.05$
0.125	14	68	$\chi^2_1 = 35.6$ $p < 0.05$

Effect of intensity (4x2 contingency table) $\chi^2_1 = 13.4$ $p < 0.05$

(duplication of MacDowall's method) there were more whiteflies on the window when it was illuminated by 550nm than 400nm and it appeared that this occurred because the whiteflies took off from the window after having landed when it was illuminated by 400nm, whereas they did not take off if it was illuminated by 550nm. The following experiments were designed to test this hypothesis and the hypothesis outlined earlier that different behaviour patterns with different spectral sensitivities may have been measured by MacDowall (1972) (compared with "slow" phototaxis - see Chapter 3).

4.6 Comparison of Two Different Methods

Two slightly different methods were developed to measure phototactic activity. One involved trapping the insects on an illuminated ground-glass surface, the other involved photographing the whiteflies on the illuminated ground-glass surface two minutes after they were introduced into the test container. The use of the two methods enabled two responses to be separated, one involved mainly initial attraction towards the lights while the whiteflies were in flight, the other involved attraction while in flight, landing and take-off behaviour over a period of time.

4.6.1 Experiment 5. Trapping on a Sticky Surface

A cylindrical container of clear plexiglass, 300mm long by 90mm in diameter was constructed. At one end were two ground-glass surfaces smeared on the inside with paraffin oil. One was illuminated with 400nm light, the other with 550nm. This was achieved by shining a beam of light from a Xenon light source onto two interference filters

mounted on the front of the container (see Figure 4.6). The light intensity was adjusted with Kodak Wratten neutral density filters and measured with the thermopile detector inside the container, 5mm from the ground-glass surface. A separate measurement was made for each illuminated surface. The intensities were those used in Experiment 4 (Section 4.5), i.e. 9.07×10^{13} quanta $cm^{-2}sec^{-1}$ and 0.5, 0.25 and 0.125 of this level. Approximately two hundred one-day-old whiteflies were dark adapted for fifteen minutes in a vial and then introduced into the container while the light was on, by gently tapping the bottom of the vial. The number of whiteflies trapped in the oil was counted after two minutes. The statistical analysis was identical to that in Experiment 4 (Section 4.5).

The results are summarised in Table 4.3. Significantly more whiteflies were trapped on the 400nm surface than the 550nm illuminated surface for all light intensities, but the proportion on the 400nm illuminated surface decreased as intensity decreased.

4.6.2 Experiment 6. Photography

The above experiment was repeated with the modification that no oil was used. Instead, the number of insects on each monochromatic surface was recorded photographically two minutes after introduction.

The results are summarised in Table 4.4. There were significantly more insects on the 550nm surface than the 400nm illuminated surface at all intensities, but the proportion on the 550nm illuminated surface fell as intensity decreased. Thus by a simple change in technique, i.e. trapping *vs* photography, completely different results were obtained.

From some general observations of whitefly behaviour, it appeared that the different results may be due to the differing activity of *Trialeurodes vaporariorum* adults under different conditions of illumination. This was supported by the results obtained in the walking and take-off experiment (Section 4.4), where the whiteflies both walked faster towards a 400nm light and were more likely to take off than when compared with 550nm. The two experiments probably measured different behavioural responses. The trapping method (Experiment 5) probably measured mainly initial attraction while the white flies were in a locomotory phase of behaviour. On the other hand, the photographic method recorded mainly the insects that alighted; it measured the same type of behavioural response as that involved in food plant selection, namely, landing after an initial take-off. The response measured with the photographic method could thus be termed a "settling" response, analogous to settling on a food plant. In Experiment 6 (photographic method) there was a population of insects in the container with a proportion of that population on one or other of the monochromatic surfaces, a proportion in flight and the rest settled on the inner surface of the container. The number on each surface would have been a result of the probability of flight towards each wavelength of light, the probability of landing on each surface (but see Section 4.9), and the probability of take-off (and walk-off) once landed. The numbers on each surface would also have depended on the proportion of the population in flight and this would have changed over the time course of the experiment. Immediately after release, most of the insects would have been in flight, but the proportion in flight falls until eventually an equilibrium is reached (see Section 4.7 and Figure 4.7). Experiment 4 (Section 4.5) has shown that while in flight, *T. vaporariorum*

is attracted more towards 400nm light than a 550nm source of light at equal quantum flux over the fairly narrow range of flux densities used. Experiment 3 (Section 4.4) has shown that the whiteflies are more likely to take-off and walk faster when illuminated by 400nm than 550nm at a single quantum flux level, i.e. they are more active under 400nm illumination. However, these results do not necessarily apply to the apparatus used in the experiments using the trapping and photographic techniques (see Figure 4.7) because the visual field was different. The light intensities and contrasts varied considerably in this container whereas there was little variation in the walking and take-off experiment (Section 4.4) and the flight orientation experiment (Section 4.5). These factors may have influenced the results since Fischbach (1979) has shown that different apparatus can give different results in "slow" phototaxis of *Drosophila melanogaster*. To attempt to explain the difference between the results of the two methods (trapping *vs* photography), and also to test the validity of the conclusions from Experiment 3 (walking and take-off) and Experiment 4 (flight orientation) using the container used in the previous experiments (see Figure 4.6), the numbers of whiteflies on the surfaces and the behaviour of individual insects in the container were observed over the whole two minute period.

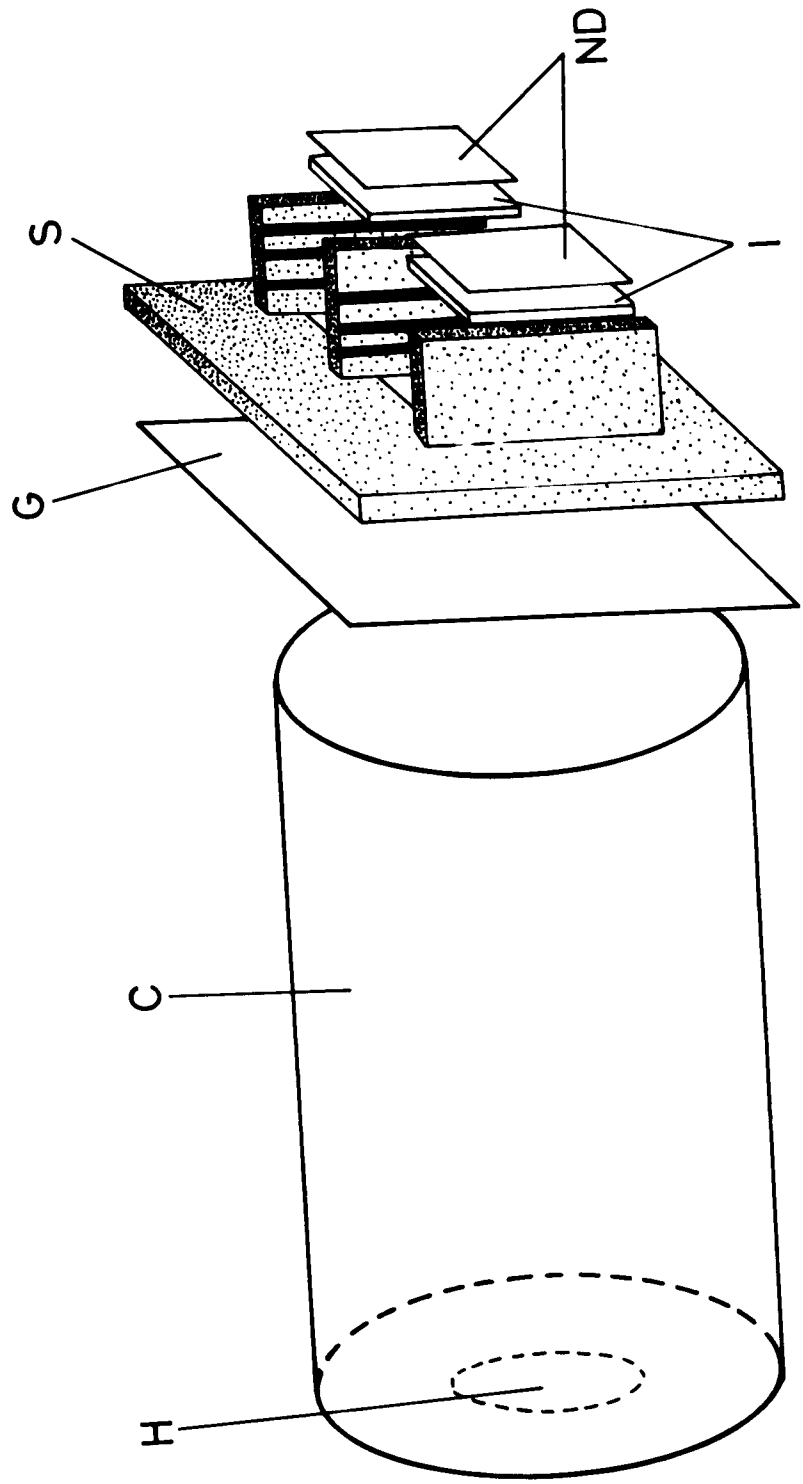
4.7 Experiment 7. Numbers of Whiteflies on the Surface with Time

The aim of the following experiment was to test the hypothesis that the difference between the results of Experiments 5 and 6 (trapping method and photographic method) was due to a change in the number of whiteflies on the surfaces, probably caused by a change in the number

of insects in flight during the time course of the experiment. If this hypothesis is true, one would expect that the numbers of insects on a surface illuminated with 400nm monochromatic light would increase rapidly at first because most of the whiteflies would be in flight and would orient towards 400nm (from Experiment 4). The numbers would then fall since the whiteflies tended to take off when illuminated by 400nm (from Experiment 3). The numbers on the surface illuminated by 550nm would probably remain static or increase gradually because orientation towards 550nm while in flight was less (Experiment 4) and the whiteflies were also less likely to take-off (Experiment 3). This result would also support the hypothesis that the results of Experiments 3 (walking and take-off) and 4 (flight orientation) were valid in Experiments 5 and 6 (trapping technique and photographic technique using the apparatus illustrated in Figure 4.6).

The container described in Section 4.5.1 (see Figure 4.6) was used. One ground-glass surface was illuminated with equal quanta of varying wavelengths (9.07×10^{13} quanta cm^{-2} sec^{-1}), the other was not illuminated. The light source was a Bausch & Lomb high intensity grating monochromator with a visible grating and Xenon light source. Appropriate Kodak Wratten gelatin filters, with the gelatin film cemented between two pieces of glass, were used to absorb higher order wavelengths. Light intensity was measured with the thermopile detector 5mm from the ground glass and was adjusted with Kodak Wratten neutral density filters. Approximately two hundred dark-adapted whiteflies (one-day-old) were introduced into the container with the light on. The container was then vigorously shaken and the number of whiteflies on the illuminated

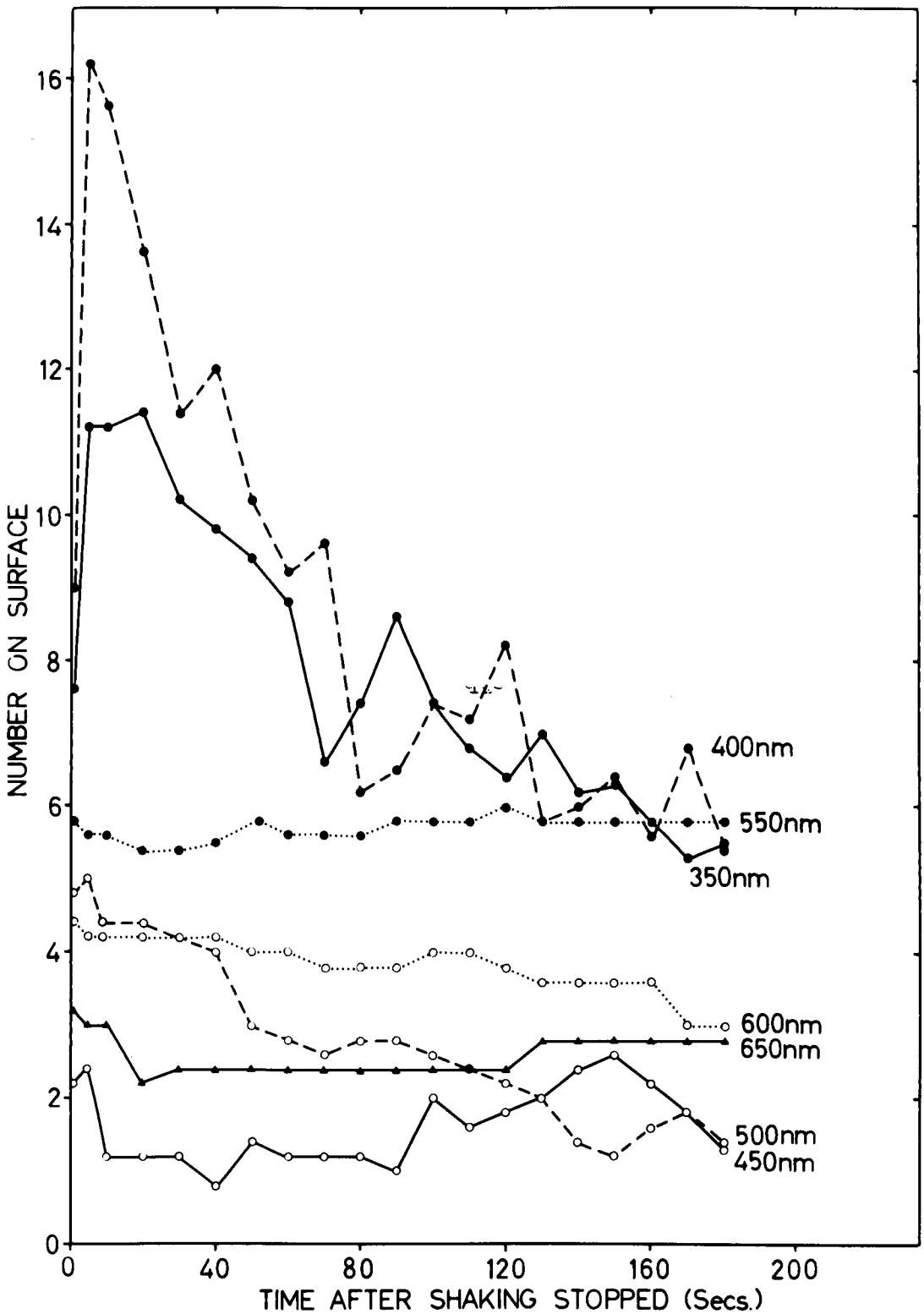
Figure 4.6 Diagram of the container used in Experiment 5 (trapping on a sticky surface), Experiment 6 (photography) and the "settling" response in Chapter 4 (without the filters). C, clear plexiglass cylinder 300mm long by 90mm in diameter; H, hole through which the whiteflies were introduced and through which a photograph was taken; G, ground-glass screen; S, light shield painted matt black; I, interference filters; ND, neutral density filters.


ND
I
S
G
C
H

surface recorded photographically, using the monochromatic light to expose the film. A photograph was taken immediately the container stopped shaking, 5 sec. after, 10 sec. after, and then once every 10 seconds until three minutes after the shaking had stopped. This procedure was repeated five times for each wavelength, with the order of wavelengths randomised.

The results show a large peak in the numbers on the illuminated surface after shaking stopped for 350nm and 400nm (Figure 4.7). The curves for the other wavelengths remained relatively stable. Thus there was a rapid accumulation of whiteflies immediately after shaking at the shorter wavelengths. It was not possible to photograph while introducing the whiteflies, since the hole through which the whiteflies were introduced was also the hole through which the photographs were taken. The shaking technique, however, probably represented a reasonable imitation of the disturbance produced when the insects were introduced into the container in Experiments 5 and 6, but it was not possible to record any accurate starting point. This is probably why the curves in Figure 4.7 began at different points. Nevertheless, the results have shown that when the whiteflies were disturbed by shaking, forcing many into flight, there was a large build-up in numbers on the short wavelength illuminated surfaces, immediately after the disturbance. The trapping technique (Experiment 5) would have recorded this initial burst of activity whereas the photographic technique (Experiment 6) would have recorded the numbers at close to equilibrium. It is also likely that the conclusions from Experiments 3 (walking and take-off) and 4 (flight orientation) were valid.

Figure 4.7 Number of whiteflies on a surface illuminated with monochromatic light plotted against time after shaking stopped. Each point is the mean of five replicates.


NUMBER ON SURFACE
0
2
4
6
8
10
12
14
16
0
40
80
120
160
200
TIME AFTER SHAKING STOPPED (Secs.)
400nm
550nm
350nm
600nm
650nm
500nm
450nm

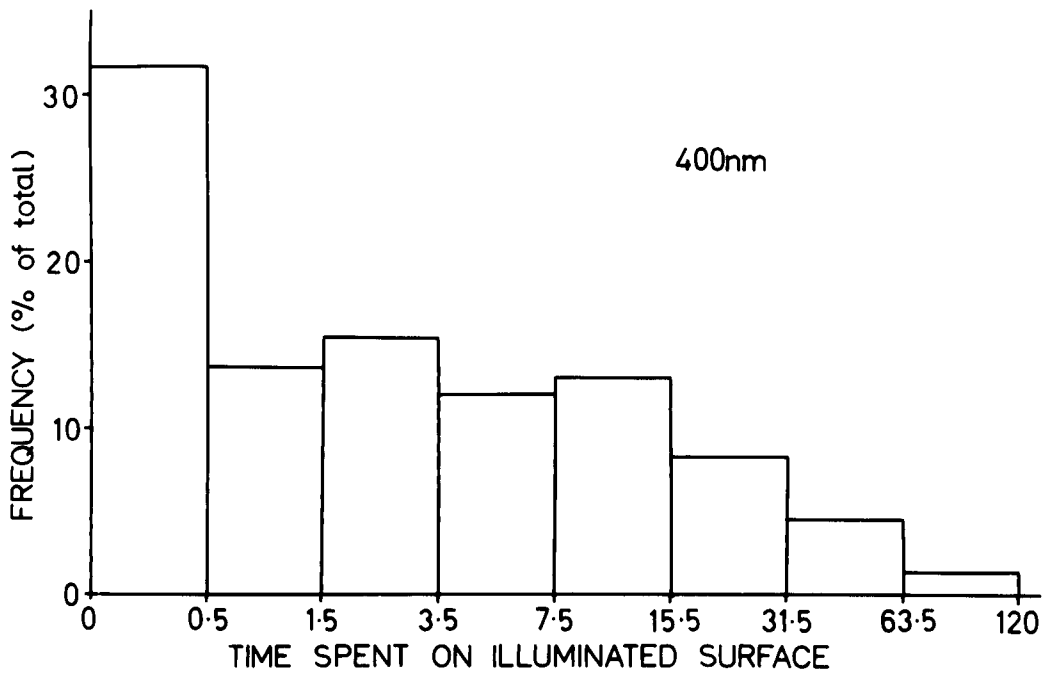
4.8 Experiment 8. Observations of Individual Whiteflies

Although the previous experiment indicated that the conclusions of Experiments 3 and 4 (i.e. whiteflies walked faster towards a 400nm light, were more likely to take off when illuminated by 400nm, and flew towards a 400nm light when compared with a 550nm light of equal quantum flux), were probably valid, the evidence supporting this supposition was indirect. The following experiment was designed to measure walking, landing, and take-off, directly, by observing the behaviour of individual insects in the container illustrated in Figure 4.6.

The container shown in Figure 4.6 was used in this experiment. One ground-glass surface was illuminated with 400nm light, the other with 550nm light (9.07×10^{13} quanta cm^{-2} sec^{-1}). A beam of light from a Xenon light source was shone on to interference filters mounted on the front of the container as in Section 4.5.1. Individual one-day-old whiteflies were dark adapted for at least fifteen minutes in a small glass tube. These individuals were then introduced into the container and their behaviour observed over a two minute time interval from the time they first took off. A description of the behaviour was recorded on a tape recorder and later transcribed to graph paper. A total of two hundred individuals was used.

A summary of the results appears in Figure 4.8 and Table 4.5. Of the two hundred individual whiteflies, 97 landed on one or other (or both) surfaces, 76 landed on the 400nm surface and 45 landed on the 550nm illuminated surface. The distribution of the length of time spent on the surfaces is shown in Figure 4.8. The frequencies

Figure 4.8 Frequency distribution of the length of time (sec.) spent on the surfaces illuminated with 400nm or 550nm monochromatic light. The frequencies have been converted to percentages so that a direct comparison can be made between the two wavelengths.


400nm
FREQUENCY (% of total)
30
20
10
0
0
0·5
1·5
3·5
7·5
15·5
31·5
63·5
120
TIME SPENT ON ILLUMINATED SURFACE

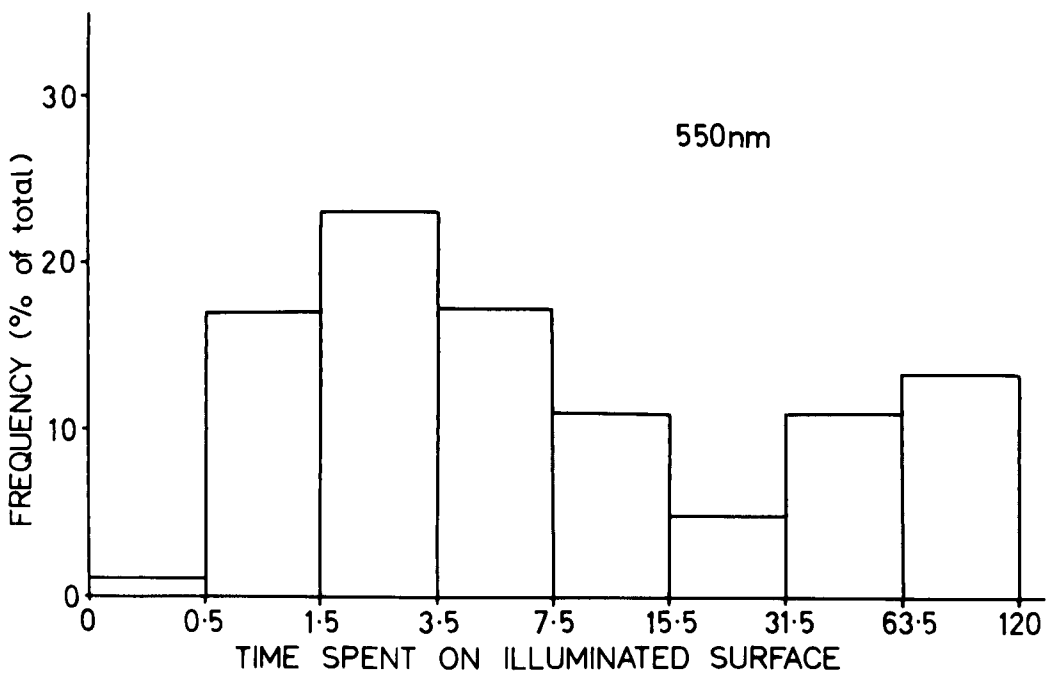

550nm
FREQUENCY (% of total)
30
20
10
0
0
0·5
1·5
3·5
7·5
15·5
31·5
63·5
120
TIME SPENT ON ILLUMINATED SURFACE

Table 4.5 Whitefly Activity.

Proportion of time spent either walking or resting on surface.

% of Time Spent	Wavelength of Illuminated Surface	
	400nm	550nm
walking	93.7	3.9
stationary	6.3	96.1

were converted to percentages so that a direct comparison could be made, since more information was obtained for the 400nm illuminated surface. The difference in the frequency distribution is significant (2x8 contingency table analysis of the original frequencies, $\chi^2_7 = 64.9$ $p < 0.05$). More whiteflies stayed longer on the 550nm illuminated surface and a significant proportion stayed longer than one minute. On the 400nm illuminated surface, the largest proportion remained for less than 0. 5 of a second.

From Table 4.5, 97% of the time spent on the 400nm illuminated surface was spent walking whereas on the 550nm illuminated surface 96% of the time the insects remained stationary. There was no evidence to suggest that the insects were going from one surface to another, i.e. the probability of landing on one surface was independent of whether the insect had been on the other surface or not. In addition, an individual was active on the 400nm illuminated surface regardless of whether or not it had previously landed on 400nm or 550nm and *vice versa*.

4.9 Experiment 9. Cessation of Wing Movement and the "Fall Reflex"

The experiments described so far have measured walking, flight, and take-off behaviour but have provided no data on landing behaviour, mainly because of the difficulty of separating orientation towards a light source of a free flying insect and landing behaviour. This may be achieved in a flight chamber (Kring, 1966) since it is possible to quantify flight by measuring rate of climb, but attempts to use a flight chamber with *Trialeurodes vaporariorum* failed (see Section 4.10). Landing behaviour is an important component of the settling and dispersive behaviour of whiteflies and needed to be examined. Moericke *et al.*'s

(1966) method of tethering a whitefly to a pin enabled landing behaviour to be measured directly and was used in the following experiment.

Moericke *et al.* (1966) found that a yellow card caused a cessation of wing movement and a "fall reflex" when it was inserted beneath a whitefly but it did not stimulate this behaviour when it was inserted above or in front of the whitefly. In Moericke *et al.*'s experiments the insects were tethered to a pin by the prothorax and were flapping their wings as if in flight, but cessation of wing movement and the "fall reflex" may well have been a landing response or a prelude to landing. Since Moericke *et al.* did not find such a response to the yellow card in front of the insect, the "fall reflex" probably did not occur in the apparatus used in the previous four experiments, because here too the illuminated surfaces were vertical. The possibility existed therefore, that in the previous four experiments, the whiteflies landed only because they were forced to when confronted with a surface (the ground-glass).

The aim of the following experiment was to test whether a landing response occurs in response to either 400nm or 550nm monochromatic light shone from below. It would have been desirable to test whether the "fall reflex" occurs in response to the light shone from in front and above the insect, as well as below, but this was not possible because of insufficient time and difficulties with the technique. The other main aim of the experiment described below was to determine the feasibility of using the "fall reflex" in further studies on wavelength specific behaviour and colour vision. Since the "fall reflex" occurred only in response to yellow and not to a series of 20 grey papers ranging from black to white (Moericke *et al.*, 1966), this response appeared to be most suitable for further studies.

The experimental apparatus is illustrated in Figure 4.9. The light source was a Xenon arc lamp. A beam splitter split the light from the lamp into two beams of white light. One beam acted as a light source for a Bausch & Lomb high intensity grating monochromator with a visible grating. The monochromatic light produced from the monochromator was reflected up onto a 90 by 100mm piece of ground glass, 5 to 7mm below a mounted whitefly. The other beam was reflected at 45^{o} above and in front of the whitefly with a surface coated aluminium mirror. The intensity of the white light was 50 μW cm^{-2} and the monochromatic light (400nm and 550nm, one half bandwidth of 10nm) $9.07x10^{13}$ quanta $cm^{-2}sec^{-1}$. Light intensity was measured with the thermopile detector at the position of the insect. The whitefly was illuminated by white light from above and monochromatic light from below in order to simulate what occurs in the field.

One-day-old whiteflies were collected and cooled in a container immersed in ice. Individual insects were attached by the prothorax onto micropins with "Clag" clear gum, after which the pin was mounted with the whitefly horizontal and approximately 5mm above the ground glass. The experimental procedure was to observe the insect in the white light only, and if it spontaneously (or when it was gently blown on) flapped its wings as if in flight for at least thirty seconds, the monochromatic light was turned on. The behaviour of the whitefly was observed for the next ten seconds, after which the monochromatic light was turned off and the procedure repeated, alternating the wavelength after every other trial. Most insects were tested at least twice and some flew long enough to be tested at the two wavelengths. If the insect did not keep flapping its wings continuously after the monochromatic light was turned off it was discarded.

Figure 4.9 Schematic representation of the apparatus used to measure the "fall reflex". X, Xenon light source; B, beam splitter; M, monochromator; S_e, entrance slit; S_x, exit slit; C, quartz-fluoride achromatic condenser; Al, aluminium surface-coated mirror; G, ground-glass screen; W, whitefly mounted on a pin; P, plasticine.

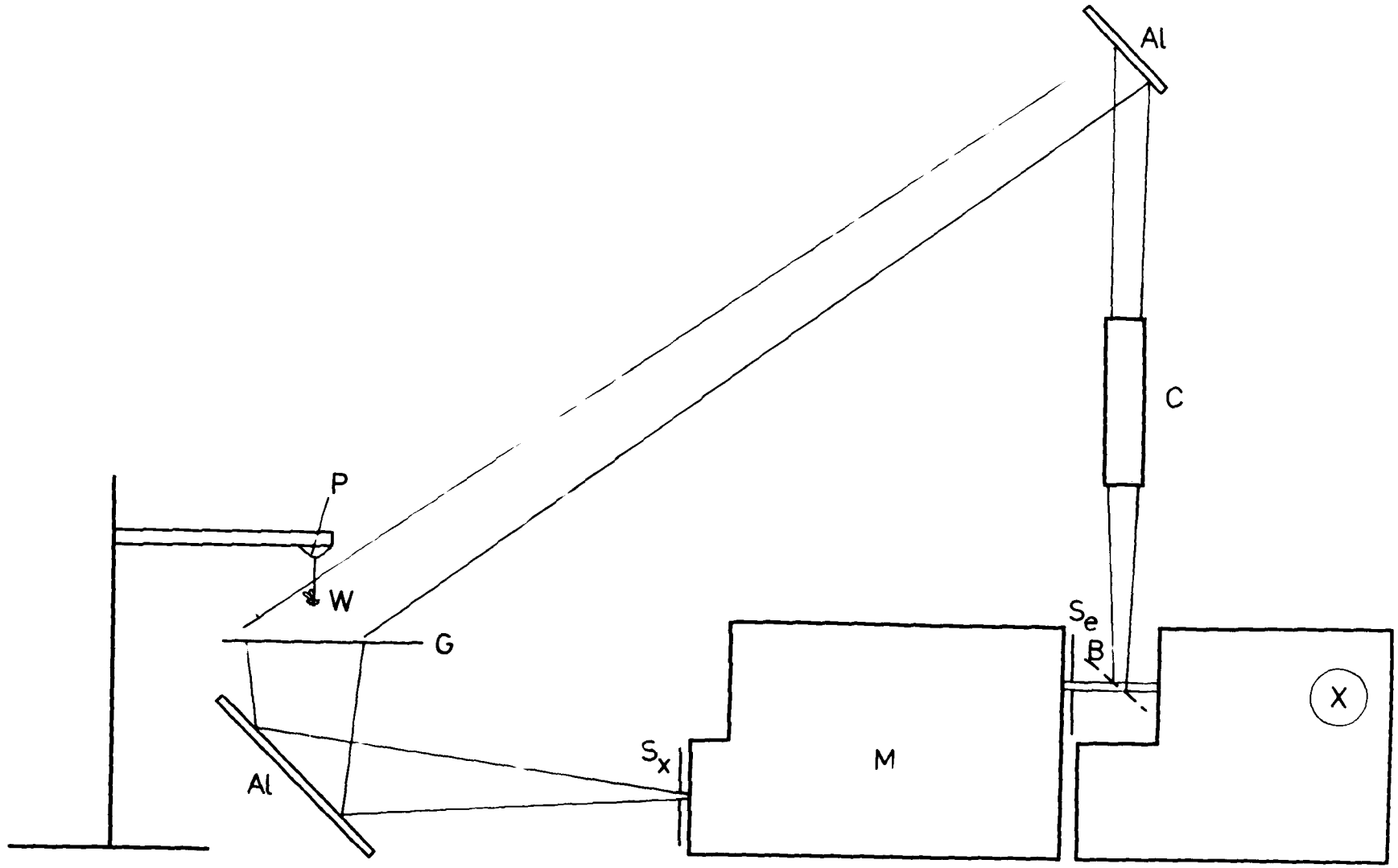

Al
C
P
W
G
Se
B
X
Sx
M
Al

Table 4.6 Cessation of Wing Movement and the "Fall Reflex".

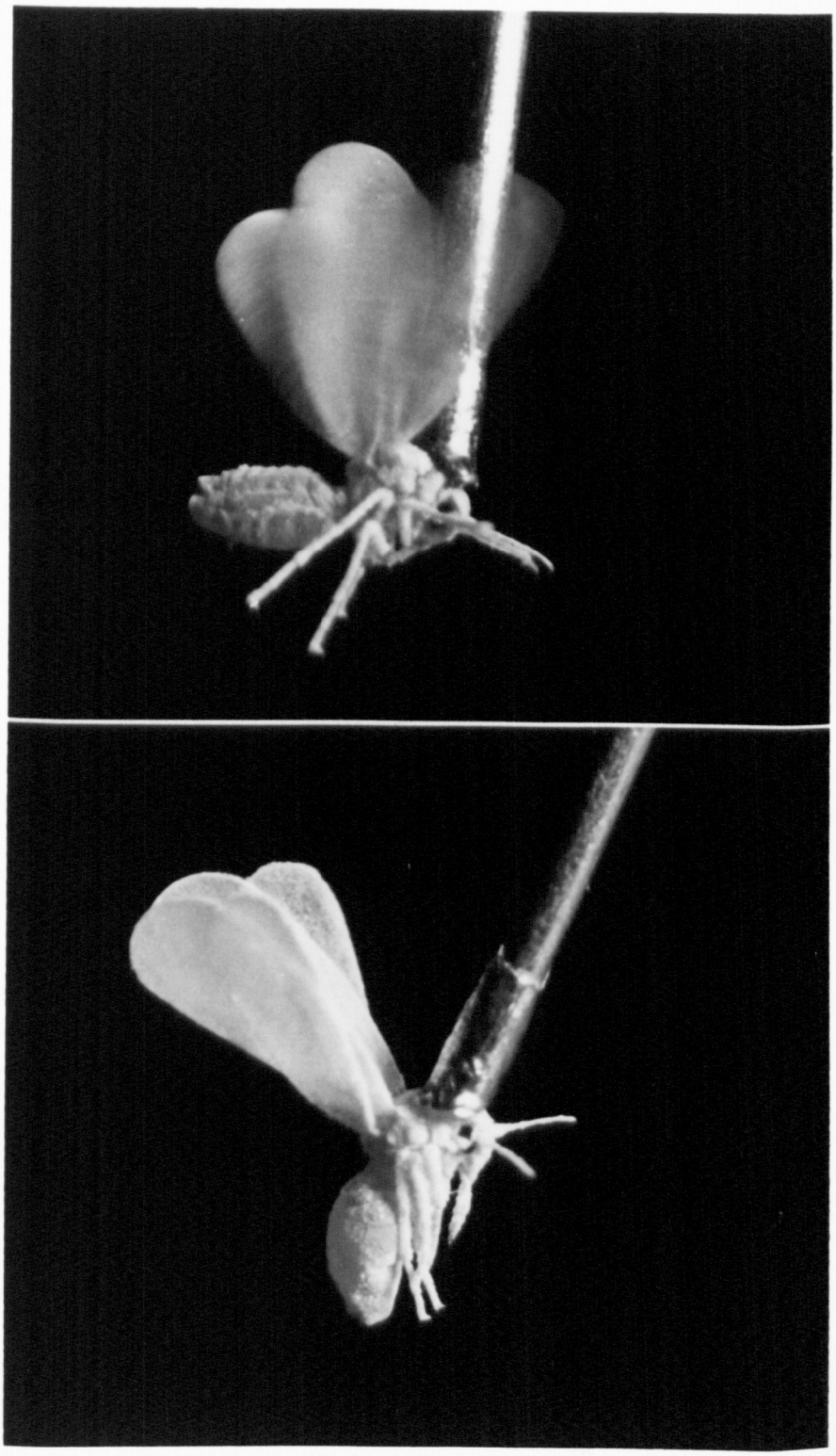
	400nm	550nm	Total
No. insects tested	13	10	20
Total No. of Trials	27	27	54
No. of Trials in Which Wing Movement Ceased Within 10 Sec.	2	26	28
No. of Trials in Which Wing Movement Continued During 10 Sec.	25	1	26
No. of Trials in Which the Whitefly Exhibited the "Fall Reflex"	0	12	12

The results are summarised in Table 4.6. From a total of twenty insects tested and a total of 27 trials at each wavelength, the whitefly ceased wing movement in 26 trials at 550nm, but in only two trials at 400nm. Three of the insects exhibited the "fall reflex" described by Moericke *et al.* (1966) when illuminated by 550nm but none when illuminated by 400nm. One individual exhibited this response ten times in ten trials under 550nm illuminuation but kept flapping its wings in ten trials at 400nm. The "fall reflex" is characterised by the following behavioural pattern. While in "flight" the whitefly maintains its body in a horizontal position (Plate 2). When adopting the "fall reflex" position it first stops wing movement, then moves its abdomen downwards and at the same time moves its wings up and together and its legs close to the body with the femora pointing upwards and the tibiae and tarsae pointing downwards (Plate 3). This behaviour is identical with that described by Moericke *et al.* (1966) in response to a yellow card placed below the insect.

The response described above is probably a prelude to landing. It is different from the landing response observed in flies (Goodman, 1960), in which the legs are moved outwards; but cessation of wing movement would cause the animal to descend and probably a final landing response may be stimulated by other stimuli such as tarsal contact or expansion of a visual pattern. The whiteflies were in an artificial situation; they were not in free flight, but tethered to a stationary pin and so had no movement of air and could not perceive any visual cues that may elicit a completed landing response. Moving visual cues are important in landing behaviour of flies (Goodman, 1960; Braitenberg and Taddei-Ferretti, 1966; Fernandez Perez de Talens and

Plate 2 A whitefly in the flight position. (x 40)

Plate 3 A whitefly in the "fall reflex" position. (x 40)



Taddei-Ferretti, 1970; Taddei-Ferretti and Fernandez Perez de Talens, 1973a and b) and the milkweed bug *Oncopeltus fasciatus* (Coggshall, 1971, 1972) where an expansion of a pattern in the visual field and/or a reduction in the luminous flux from the patterns stimulates a landing response. Another point which must be considered is the relatively large size of the pin. Although the finest micropin available at the time was used, the size of the pin was still large in relation to the small size of the insect. This may have interfered with its movement to some extent. Mounting the whiteflies was a very delicate procedure and many were mounted but immediately discarded because the pin or the gum hindered wing movement. This could explain why only a small proportion of the whiteflies could be made to fly continuously; but those that did, behaved consistently, whether in their "fall reflex" response to 550nm, or continuous flight in response to 400nm. This phenomenon was also observed by Moericke *et al.* (1966) who reported that only 5-20% of the insects mounted flew continuously. Because of the artificiality of the experiments discussed above, the results must be interpreted with caution; nevertheless, the fact remains that whiteflies which were flapping their wings as if in flight, ceased wing movement and sometimes exhibited the "fall reflex" in response to 550nm light from below, and they did not do so in response to the 400nm light.

This method was considered not to be suitable for further studies because it was very time-consuming due to the difficulties of mounting the whiteflies and due to the fact that only a small proportion that were successfully mounted flew continuously.

4.10 Discussion

4.10.1 Conclusions

Some general conclusions can be made from the results of the nine experiments. When the two standard wavelengths and intensities are compared, *Trialeurodes vaporariorum* is most likely to orient towards 400nm when in flight, but will tend to land on a 550nm surface. Once they land on a 550nm illuminated surface, they will remain stationary and are less likely to take off again than if they happen to land on a 400nm illuminated surface. If they land on a 400nm illuminated surface or if illuminated by 400nm, the whiteflies are likely to take off again; and if they do not take off they will walk actively. The net effect of this is an accumulation on a 550 nm surface as observed by MacDowall (1972). This is also compatible with previous observations in Chapter 3.

4.10.2 Ecological significance

The ecological significance of this behaviour is that free flying whiteflies would orient towards the sky ("open space", Mazokhin-Porshnyakov, 1969), but would tend to land on a green plant, because green plants reflect maximally at about 550nm in the visible, and absorb ultra-violet (Shull, 1929; Woolley, 1971; Vaishampayan *et al.*, 1975a). The actual host selection process probably occurs after landing, using stimuli other than visual, similar to how aphids select a food-plant (Kennedy *et al.*, 1961). This is supported by the results obtained by Vaishampayan *et al.* (1975b) and Verschoor-van der Poel and van Lenteren (1978).

The behaviour of *T. vaporariorum* is similar to that of aphids. Aphids tend to land on a yellow surface (Moericke, 1952, 1955; Kring, 1967; Posposil, 1962; Zadarek and Pospisil, 1966a, b), but fly towards shorter wavelengths (Kring, 1969). Kring (1969) found that aphids flew towards a UV fluorescent lamp, whereas a yellow lamp was relatively unattractive. Under the UV lamp, the aphids became very active and moved rapidly around the screen covering the lamp, but under the yellow light they were not active. He concluded that the yellow inhibited flight activity. These results are analogous to whitefly behaviour. However, it must be kept in mind that the intensities of light in the aphid experiments were not controlled as they were in this study and so the conclusions reached are open to question.

A great deal more is known about aphid behaviour from experiments done in a flight chamber (Kring, 1966; Kennedy and Booth, 1963a, b, 1964; Kennedy, 1965, 1966; Kennedy and Fosbrooke, 1972; Kennedy and Ludlow, 1974). For example, a flying aphid initially shows a preference for shorter wavelengths, but during a long uninterrupted flight, develops a preference for longer wavelengths. An aphid in simulated outdoor conditions (white light above, long-wave reflecting surface below) eventually flies downwards but can still be shown to be responding to the white light as well. Thus, the relative attractiveness of the incident and long-wave light reverses as the flight lengthens (Kennedy and Fosbrooke, 1972). It is unknown whether this occurs with *T. vaporariorum* because no experiments were done in a flight chamber. A flight chamber enables quantification of flight by measuring rate of climb. This means that it is possible to separate flight and landing behaviour in the same apparatus. This could not be done in any of the experiments in this study. An attempt was made to use a flight chamber, but it failed due to two main difficulties. Whiteflies would not fly when there was any

air movement, which made it impossible to suspend an insect in an air stream as has been done with aphids. The other problem encountered was that even when there was no air movement and the whiteflies flew, they did not fly upwards towards the light. The success of the flight chamber depends on this response. The failure of the whiteflies to fly towards the light may have been due to the nature of the light source (tungsten filament). With the benefit of hindsight it would have been desirable to use a Xenon lamp rather than a tungsten lamp in the flight chamber because a Xenon arc has a spectral emission spectrum similar to noon sunlight, whereas a tungsten lamp has a high content of longer wavelengths (yellow, red and infra-red) and almost no ultra-violet (Seliger and McElroy, 1965). In this study, wavelengths of 400nm and shorter (i.e. UV) have been shown to stimulate flight, and so the choice of a white light source in the flight chamber must take this into account. It would have been desirable to test the effects of different light sources in the flight chamber but there was insufficient time to do so.

The walking behaviour observed in Experiments 3 and 8 may explain a typical behaviour pattern observed in the field. When a whitefly lands on a bean plant (a favourable host) it often lands on the upper surface of the leaf. If it does, it immediately begins to walk, and keeps walking until it reaches the edge of the leaf, where it walks around the edge onto the underside. Once on the underside of the leaf it immediately stops and often probes. This behaviour may be a response to gravity but the following experiment would indicate otherwise. If a beam of light from a Xenon arc in a darkroom is shone onto the underside of a leaf with whiteflies feeding on it, many of them will remove their stylets and start walking or take off. The end

result is that most of the whiteflies come to rest on the upper side of the leaf (the non-illuminated side). The hypothesis formulated from these observations and the experiments, is that the whiteflies' response to sunlight (or the Xenon lamp) similar to their response to 400nm monochromatic light, i.e. they walk actively (c.f. Experiments 3 and 8) until they reach the underside of the leaf, but even on the upper surface flight is inhibited because of the green leaf below (c.f. Experiments 3, 8 and 9). Observations of their behaviour under illumination from a Xenon arc lamp supports this hypothesis, since they are very active and will fly strongly towards the light, even if it is shining from below. One other explanation that was not tested because of insufficient time and inadequate equipment is that they responded differently to reflected and transmitted light. However, much of this is conjecture and before any definite conclusions can be made, more work needs to be done on the various responses, especially the effect of intensity, since only one constant quantum flux level (or a limited range) was used in all the experiments in this chapter.

None of the experiments described so far and none of the data available from the literature give any information on colour vision in *T. vaporariorum*, although some does suggest wavelength specific behaviour (e.g. Moericke *et al.*, 1966; Vaishampayan *et al.*, 1975a). Because relatively few insect species have been shown to have true colour vision, and also because of the hypothesis stated by MacDowall (1972), Vaishampayan *et al.* (1975a, b) and Verschoor van der Poel and van Lentern (1978), that the first steps of food plant selection of *T. vaporariorum* occurs as a response to colour, wavelength specific behaviour and colour vision in *T. vaporariorum* was investigated as detailed in the next chapter.

CHAPTER 5

5. WAVELENGTH SPECIFIC BEHAVIOUR AND COLOUR VISION

5.1 Introduction

True colour vision has been defined as the ability of an animal to distinguish colours on the basis of differences in wavelengths only, independently of differences in brightness or the absolute spectral distribution of the stimulus (Menzel, 1979). Colour vision so defined involves central integration of the outputs of photoreceptors with different spectral sensitivities (Hu and Stark, 1977). An animal with true colour vision can distinguish between specific wavelengths with a maximum discriminative ability falling between the peak sensitivities of the organism's photoreceptors (e.g. ants (Kretz, 1979), bees (von Helversen, 1972)).

Wavelength specific behaviour, on the other hand, has been defined by Menzel (1979) as when "an animal displays markedly different $S(\lambda)$ (i.e. spectral sensitivity) for different behaviour patterns". This implies that photoreceptors with different spectral sensitivities may trigger or control different behavioural patterns, but that the outputs of these receptors are only integrated in the central nervous system in the sense that one or another of the alternative behaviour patterns is selected. It may be possible to demonstrate that a particular behavioural response is apparently intensity independent when the behavioural patterns contributing to that response are antagonistic (see Section 5.5), but in contrast to true colour vision, wavelength specific behaviour cannot be independent of the spectral distribution of the stimulus (for the purposes of this study, behavioural pattern means the behaviour controlled or triggered by a photoreceptor,

behavioural response is the resultant behaviour that is measured), i.e. the organism responds to the spectral stimuli as if they were independent stimuli, and therefore the resultant response is dependent on the absolute spectral distribution of the stimulus (and the intensity). To complicate matters, an animal with colour vision may display wavelength specific behaviour, but the converse does not necessarily follow, i.e. the presence of photoreceptors with different spectral sensitivities, or wavelength specific behaviour, does not imply that an animal has colour vision. This has to be shown behaviourally.

The most reliable method of demonstrating true colour vision is to train individuals of a species to respond in a certain manner to a particular hue (e.g. to come to feed at a feed station in response to a certain colour) and to show that such behaviour persists when the stimulus varies in brightness with respect to backgrounds of various other hues. Normally monochromatic light is used to exclude the parameter of wavelength purity ("colour saturation"). By this means it can be shown that a bee, for example, can distinguish between monochromatic wavelengths differing by as little as 4.5nm (von Helversen, 1972). In animals that cannot be trained it is much more difficult to demonstrate true colour vision. The major difficulty is in dissociating the effect of quantum flux (at various wavelengths) from effects of wavelengths. Training techniques have the advantage that they deal with discriminations *per se*, in a situation where the "motivation" to make the discrimination is strong. A training paradigm can often be designed in such a way that the training effect can only be wavelength dependent (or brightness dependent, or both) and the presence or absence of a training (or conditioning) effect which is wavelength dependent independent of brightness is a definitive result. The effect of

brightness can largely be removed, but in animals that cannot be trained, the distinction between "brightness" and "hue" will always be based on the interpretation of the behavioural measure used to quantify the effect of quantum flux. Here the distinction must be made between brightness and intensity. Intensity can be defined and measured in purely physical terms, e.g. quanta $cm^{-2}sec^{-1}$ or in energy units, $\mu W\ cm^{-2}$, but brightness is the physiological (or psychological, in human studies) analogue of intensity, and cannot be measured physically, it can only be estimated using the responses of the animal. "Brightness and hue (and saturation, if other than spectral lights are considered) are constructed values of a model of the nervous system and probable relevant values for the computation of the nervous system, but they are not dissociable parameters" (Menzel, personal communication). Although brightness and hue are related to intensity and wavelength respectively, this is not necessarily a simple relationship. Brightness will also depend on the context in which it is being estimated, because the nervous system may "weight" the inputs differently in different behavioural contexts. Brightness normally is measured subjectively in man, and in animals by monitoring behavioural responses since colour vision can only be studied by behavioural methods. In animals this is normally achieved by measuring a response at various levels of quantum flux (intensity response functions, usually a log relationship) and defining equal "brightness" as the intensities that have equal behavioural effectiveness, i.e. an "action spectrum" is an estimate of relative "brightness". However, this method is only valid if one can assume univariance for that particular behavioural response. The principle of univariance states that for a single photoreceptor, the response depends on the number of quanta absorbed, not the wavelength

(Naka and Rushton, 1966). Thus if a behavioural (or other) response is univariant, intensity response functions measured for different wavelengths will be parallel for that particular response. One example of this method is Kretz's (1979) study on colour vision of the desert ant *Cataglyphis bicolor*. If the intensity response functions are not parallel, the above definition of brightness depends on the criterion of response used. Consequently, brightness is no longer a simple parameter, i.e. there is no simple relationship between brightness and intensity, wavelength is important; brightness and hue are not dissociable. Intensity response functions often are not parallel and in these cases other methods which do not depend on estimates of relative brightness need to be used.

Among the insects the colour vision system of the honey-bee has been most studied (von Frisch, 1914; Kühn, 1927; Daumer, 1956; von Helversen, 1972; Neumeyer and von Helversen, 1976; Neumeyer, 1980). Only in the bee (von Helversen, 1972) and the desert ant *Cataglyphis bicolor* (Kretz, 1979), has colour vision been studied sufficiently to enable a spectral discrimination function to be constructed. In other insects, colour vision has been studied only qualitatively (reviewed by Burkhardt, 1964; Mazokhin-Porshnyakov, 1969; Autrum and Thomas, 1973; Goldsmith and Bernard, 1974; Menzel, 1975; Menzel, 1979). Only a few insects have been shown to have true colour vision, despite the large number of species studied. This is probably because many species are difficult to train (Mazokhin-Porshnyakov, 1964; Menzel, 1979), training being the most powerful technique available. Nevertheless it is probable that true colour vision exists in, for example, *Papilio troilus* (Swihart, 1971), *Paravespula germanica* (Beier and Menzel, 1972) and *Formica polyctena* (Kiepenheuer, 1968). All of these species

have been successfully trained. *Drosophila melanogaster* has been conditioned to blue and yellow; according to Menne and Spatz (1977), the conditioning effect was largely independent of intensity, but Bicker and Reichert (1978) found that conditioning was wavelength and intensity dependent. Simultaneous and successive colour contrast has also been demonstrated in "slow" phototactic behaviour of *Drosophila melanogaster* (Fischbach, 1979). A successive colour contrast was demonstrated in the peach aphid *Myzus persicae* by Moericke (1950) but this cannot be taken as evidence for true colour vision because receptor processes (see below) were not eliminated.

The whitefly *Trialeurodes vaporariorum* shows a spontaneous preference for yellow (Lloyd, 1922; MacDowall, 1972; Vaishampayan, 1975a; see also Chapter 4) and appeared to be a good candidate for true colour vision, since Moericke *et al.* (1966) found that the "fall reflex" was apparently intensity independent. The "fall reflex" was a reaction that occurred to yellow and was characterised by cessation of wing movement of a flying whitefly, followed by downward movement of the abdomen, movement of the wings up and together and the legs close to the body with the femora pointing upwards and the tibae and tarsae pointing downwards (see Section 4.9). The work of Moericke *et al.* (1966) and Vaishampayan (1975a) indicated that the behaviour of *T. vaporariorum* might alternatively be wavelength specific only, but the results are difficult to interpret because the role of ultra-violet light was neglected and the intensities and spectral composition of the stimuli were not sufficiently controlled.

There are many exmaples of wavelength dependent behaviour, but relatively few well analysed cases of wavelength specific behaviour (reviewed by Menzel, 1979). The term "wavelength dependent behaviour" refers to cases where a wavelength-response function cannot be regarded as a spectral sensitivity (quantum flux level for equal response *vs* wavelength) or an efficiency function (response measured at equal quantum flux *vs* wavelength) (Menzel, 1979), usually because intensity was not controlled at the various wavelengths, or it was measured using the wrong units and the spectral composition of the stimulus was not monochromatic. Wavelength specific behaviour refers to cases where it has been shown that different behavioural patterns have different spectral sensitivities. In only a few cases where wavelength specific behaviour has been demonstrated in insects, has this behaviour been studied in enough detail to provide information on the complexity of the visual system underlying this behaviour. In the case of the honey-bee and *Drosophila*, which are the only well analysed cases, in certain behavioural contexts (honey-bee - food gathering, *Drosophila* - "slow" phototaxis and conditioning using "fast" phototaxis) the system is indeed complex, and certainly in the case of the honey-bee involves true colour vision. Demonstration of wavelength specific behaviour is only the first step in a study of "colour" vision. The question one must then attempt to answer is how complex is the neuronal system controlling this behaviour?, i.e. where is that animal along the evolutionary path from "true" wavelength specific behaviour (i.e. a relatively simple system) to variously highly developed "true" colour vision systems. "True" wavelength specific behaviour patterns are especially interesting because they may help to elucidate the sensory and interneuronal mechanisms of colour vision in invertebrates (Menzel, 1979). This chapter demonstrates wavelength specific behaviour in the whitefly *Trialeurodes*

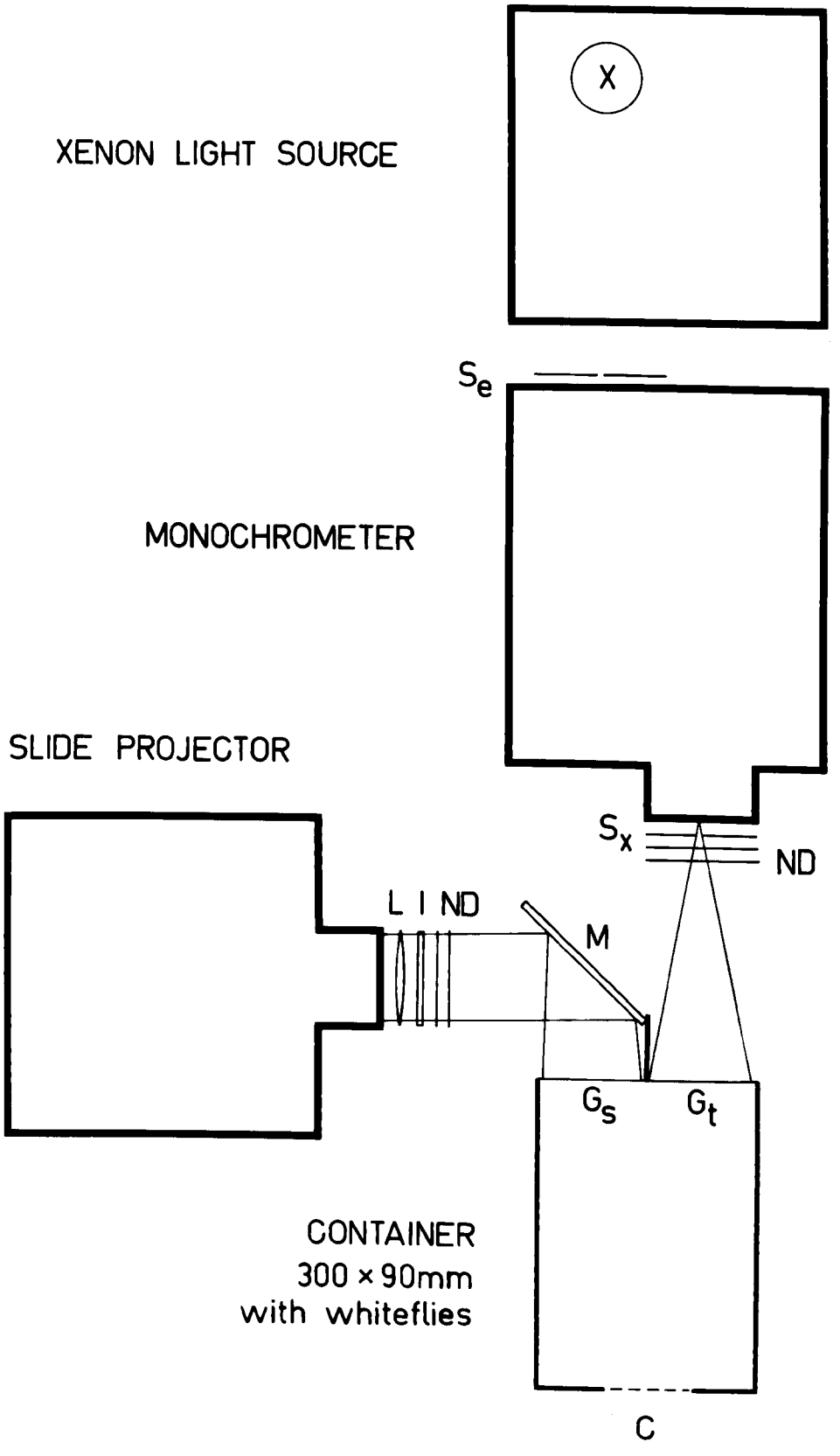
vaporariorum, using a "settling" response analogous to landing on a host plant after a short flight, and also a form of phototaxis that probably conforms to the "fast" phototaxis used by Menne and Spatz (1977). An attempt was made to determine whether "fast" phototaxis was wavelength specific only, or involved colour vision.

5.2 Intensity Response Functions of the "Settling" Response

The aim of the following experiment was to determine intensity response functions at various wavelengths for the "settling" response using the photographic technique (Section 4.6.2). This method was employed because it was the most reliable and least laborious of all the methods developed at the time, and was analogous to the whiteflies landing on a host plant after a short flight.

The apparatus used in the experiment described below is illustrated in Figures 4.7 (without the filters) and 5.1. The test container (Figure 4.7) consisted of a clear plexiglass cylinder 300mm long, 90mm diameter. At one end were two vertical ground-glass surfaces, one illuminated with a constant intensity of 400nm light (standard), the other with varying intensities and wavelengths (test). The two surfaces were separated by a vertical divider to prevent leakage of light from one surface to another. The positions of the standard and test lights were alternated and the container cleaned with distilled water between each trial. A black screen was placed around the container to eliminate a small amount of stray light from the light source. All trials were carried out in a darkroom maintained at $25^{o}C \pm 1^{o}C$ and a relative humidity of 50-60%.

Figure 5.1 Diagrammatic representation of the apparatus used to measure the "settling" response. X, 150W Xenon lamp; S_e, entrance slit; S_x, exit slit; L, lense; I, interference filter; ND, neutral density filters; M, aluminium surface coated mirror; G_s, ground-glass screen illuminated by the test light; C, hole through which the whiteflies were introduced into the container and through which a photograph was taken of the whiteflies on the ground-glass screens two minutes after the whiteflies were introduced.


X
XENON LIGHT SOURCE
S_e
MONOCHROMETER
SLIDE PROJECTOR
S_x
ND
L I ND
M
G_s
G_t
CONTAINER
300 × 90mm
with whiteflies
C

The light source for the standard light was a slide projector fitted with a 400nm interference filter with a one-half bandwidth of 10nm (Oriel G-574-4000). Monochromatic light from this projector was reflected onto the ground-glass via an aluminium surface-coated mirror. The light source for the test light was a Bausch & Lomb high intensity grating monochromator with a visible grating (33-86-02) and Xenon lamp (except for the highest intensity at 660nm, when the projector fitted with a 660nm interference filter and a Wratten filter No. 25 was used as the test light source, with the monochromator as the standard light). The slit widths were adjusted to give a one-half bandwidth of 10nm (except for the highest intensity at 350nm when the bandwidth was 20nm), and the monochromator was fitted with appropriate cut-off filters (Kodak Wratten filters Nos. 2B, 16 or 25) to eliminate higher order wavelengths. Light intensity was adjusted with Kodak Wratten neutral density filters. Both the cut-off filters and the neutral density filters were used with the gelatin film cemented between two pieces of glass. The flux of the standard light and higher intensities of the test light were measured 5mm from the ground-glass, inside the testing container, with a Reeder thermopile and Keithley 249 milli-microvoltmeter. A separate measurement was made for each surface. Lower intensities, below the usable sensitivity of the thermopile, were calculated from the optical densities of the neutral density filters which had previously been calibrated at appropriate wavelengths.

Approximately 200 whiteflies were dark-adapted for fifteen minutes in a polystyrene vial (8.3x3.5cm) placed in a light-proof box; they were then introduced into the container, through a hole in the end opposite to the ground-glass, by gently tapping the vial containing the insects.

The insects were introduced while the lights were on. The number of whiteflies on each surface was recorded photographically after two minutes. The "settling" response (SR) was represented by a percentage -

$$SR = \frac{N_t}{N_s + N_t} \times 100$$

where N_s is the number of insects recorded on the standard (400nm) surface and N_t the number recorded on the test surface.

Means and standard errors are given. The action spectrum and the standard errors were calculated from a linear regression of the linear parts of the intensity response curves; except for 660nm where the quantum flux level which produced a 50% response was read directly from the graph of means (Figure 5.2). Analysis of the experiments on wavelength specific behaviour (Table 5.1) and the effect of light adaptation (Table 5.2) was by analysis of variance with a log transformation to stabilise the variance.

The results are shown in Figure 5.2. Intensity response functions were determined for seven wavelengths (Figure 5.2). Since the shapes of the curves were different for different wavelengths, univariance (Naka and Rushton, 1966; Rodieck, 1973; Menzel, 1979) could not be assumed for this behavioural response. Thus, if univariance holds for single photoreceptors, and there is evidence to suggest that it does hold for several invertebrate visual cells (Järhvilehto, 1979), at least two photoreceptors must be involved in this behavioural response. The action spectrum for the 50% response level (Figure 5.3) showed a peak at 450nm and rising values into the ultra-violet. MacDowall (1972) constructed a spectral efficiency function by measuring the number of

Figure 5.2 Intensity response functions. "Settling" response plotted against log relative intensity. The "settling" response was expressed as a percentage of the total number of whiteflies, recorded on the surface illuminated by the test light. Log intensity = zero represents 15.9×10^{13} quanta $cm^{-2} sec^{-1}$. The response to darkness is (400 nm standa
vs darkness) is also shown (-----). The vertical lines represent standard errors of the mean of ten replicates. The response to darkness was measured each time the response to a different wavelength was tested (one wavelength was tested per day). There were no significant differences between any of the dark responses.

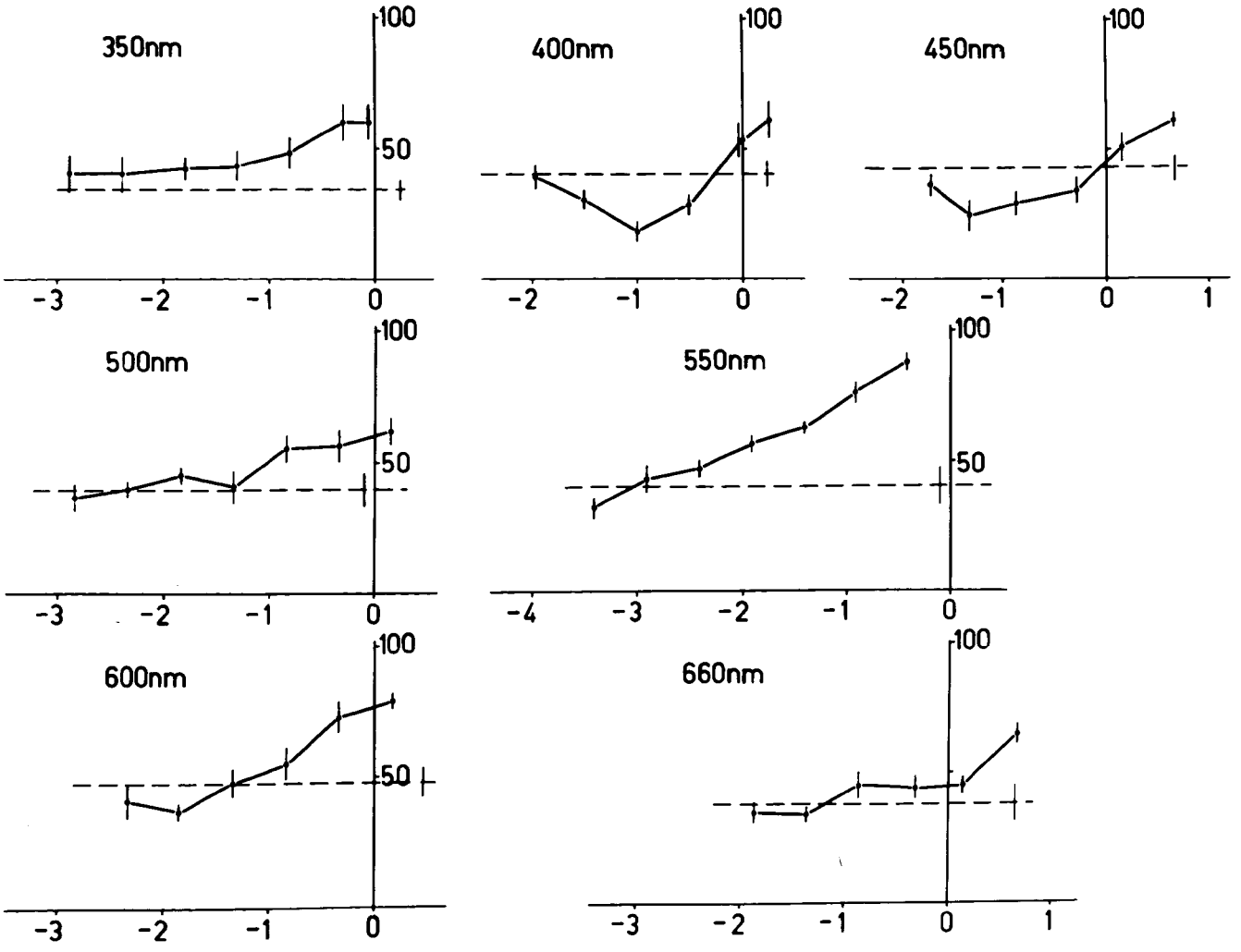
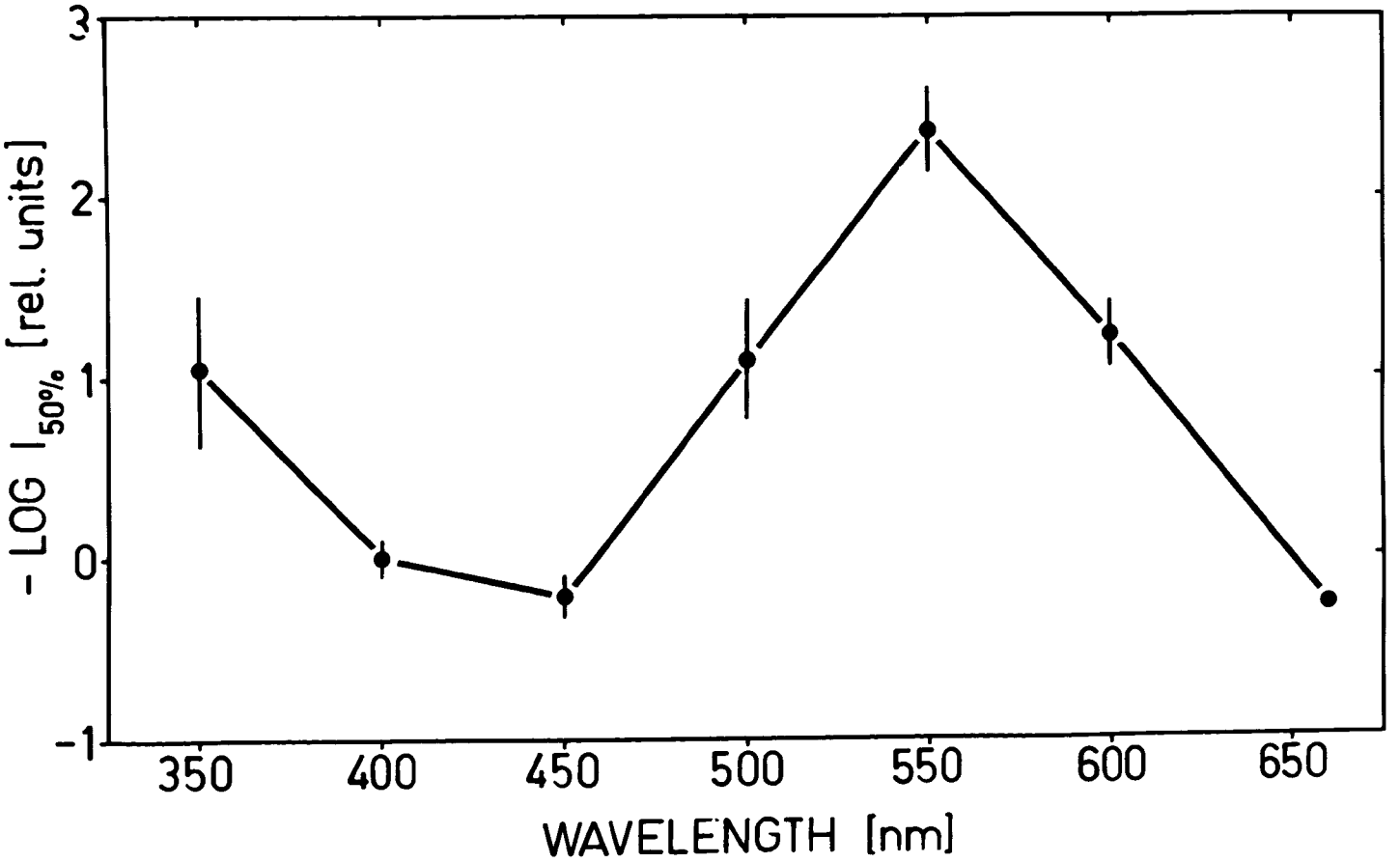

350nm
100
50
-3
-2
-1
0
400nm
100
-2
-1
0
450nm
100
-2
-1
0
1
500nm
100
50
-3
-2
-1
0
550nm
100
50
-4
-3
-2
-1
0
600nm
100
50
-3
-2
-1
0
660nm
100
-3
-2
-1
0
1

Figure 5.3 Action spectrum of the "settling" response. Minus the log of the intensity which produced a 50% response (from a regression of the linear parts of the intensity response functions (Figure 5.2)) plotted as a function of wavelength. The 50% response represents equal numbers on the test and standard surfaces. The intensity which produced a 50% response was calculated from the regression equation.


3
2
1
0
-1
- LOG $I_{50\%}$ [rel. units]
350
400
450
500
550
600
650
WAVELENGTH [nm]

whiteflies landing on a window illuminated with equal quanta of monochromatic light of various wavelengths, a response similar to the "settling" response. The action spectrum of the "settling" response agrees with MacDowall's (1972) spectral efficiency function which peaked at 550nm and closely followed the transmission spectrum of a tobacco leaf (475nm - 625nm), but since the "settling" response was not univariant, the shape of the action spectrum changes with response level and thus equal "brightness" cannot be defined as the intensities that produce an equal response, and so the action spectrum is not useful for colour vision studies. This experiment provides no evidence for colour vision *per se*, but does indicate that wavelength as well as intensity provide important behavioural cues. There is no simple relationship between "brightness" (as defined above) and intensity, wavelength also must be considered. The whiteflies were probably exhibiting wavelength specific behaviour.

The behavioural patterns involved in the "settling" response involved flight towards the lights, landing and take-off behaviour. It is likely that short wavelengths ($\leqslant$ 400nm) stimulate flight and inhibit landing of *T. vaporariorum*, while longer wavelengths stimulate landing and inhibit flight (see Chapter 4). To elucidate one of the possible behaviour patterns which may be involved in the "settling" response, and also to show that *T. vaporariorum* shows wavelength specific behaviour according to Menzel's (1979) definition, the test paradigm was slightly modified. The ground-glass screen was smeared with a layer of paraffin oil which trapped all the whiteflies that landed on it. By comparing this method with the photographic method which measured the "settling" response, one possible behaviour pattern contributing to the overall "settling" response could be separated.

Table 5.1 Photographic Method *vs* Trapping Method.

Wavelength	Percentage of the total on the Test Surface (mean of ten replicates)		
	Photographic method ("settling" response)	Trapping method	
350nm	54.4	67.1	n.s.
450nm	48.9	11.8	**
550nm	50.0	20.2	**
	n.s.	**	

** - Significant at the 1% level.

n.s. - Non significant.

Table 5.2 Effect of Light and Dark-Adaptation. Tested at 550nm.

	Percentage of the Total on the Test Surface (mean of ten replicates)		
	Photographic method ("settling" response)	Trapping method	
Light adapted	50.7	25.7	**
Dark adapted	55.8	18.8	**
	n.s.	n.s.	

** - Significant at the 1% level.

n.s. - Non significant.

This component was the initial attraction to an illuminated surface while the whiteflies were in a locomotive phase of behaviour.

At the intensity that gave a 50% "settling" response (from Figure 5.2), at 350nm, 450nm and 550nm the response using the paraffin was markedly different from the "settling" response measured by photography (Table 5.1). The response to 450nm and 550nm was much less when the trapping method was used and so the action spectrum for the trapping paradigm is different from the photographic paradigm. This might imply different underlying behaviour patterns with different spectral sensitivities; but the data could also be explained by assuming that the two methods recorded the effects of the one basic behavioural response but in insects at different stages of light adaptation. Thus, in the trapping paradigm many of the whiteflies were trapped soon after they were introduced and so were probably dark-adapted, whereas in the "settling" response paradigm the whiteflies were probably light-adapted. To test whether the adaptation levels could have influenced the observed responses, groups of whiteflies were therefore either dark-adapted or light-adapted in a beam from a Xenon arc lamp for fifteen minutes before being tested with one or other of the paradigms.

From Table 5.2, it can be seen that, at least for the 550nm light, light-adapted and dark-adapted whiteflies behaved similarly. *T. vaporariorum* clearly shows wavelength specific behaviour (attracted to violet, settling on green) and this raises the possiblity that they may have true colour vision.

Training techniques are by far the most reliable techniques for studying colour vision and so an attempt was made to develop a training paradigm for *T. vaporariorum*. *Drosophila melanogaster* has been successfully conditioned to visual stimuli using shaking as an aversive conditioning stimulus (Menne and Spatz, 1977; Bicker and Reichert, 1978; Reichert and Bicker, 1979) and the method described in the next section was based on this technique.

5.3 Conditioning

Drosophila melanogaster and *Musca domestica* have been successfully conditioned to visual stimuli (Quinn *et al.*, 1974; Spatz *et al.*, 1974; Menne and Spatz, 1977; Bicker and Reichert, 1979; Reichert and Bicker, 1979) and are the only species, apart from hymenoptera (see Section 5.1) and a few butterflies (Swihart, 1971), that have been conditioned to brightness and/or wavelength of light. In the experiment described below, the method used by Bicker and Reichert (1978) was slightly modified, taking into account the small size of *T. vaporariorum* and the observation that *T. vaporariorum* is much less active than *Drosophila* under the same conditions. This method had the advantage that the training procedure was symmetrical, which enabled the influence of the conditioning stimulus alone on the phototactic behaviour to be eliminated. The method also proved to be much less tedious and more reliable than the method used to measure the "settling" response. For this reason it was used in all further experiments. However, the method is a measure of phototaxis, probably "fast" phototaxis (Menne and Spatz, 1977), and bears no relation to food-plant selection (unlike the "settling" response).

Two automatic slide projectors (Figure 5.4), fitted with interference filters (400nm and 550nm, one-half bandwidths of 10nm), were used as light sources. One projector, fitted with the 550nm filter, was also fitted with Wratten filter No. 4 in order to eliminate higher order wavelengths. The projectors were focussed on the test container; slides, which were clear or blacked-out on one side were exchanged in the projectors according to the illumination desired. The difference in light intensity between the illuminated side of the test container compared with the non-illuminated side was of the order of two log units. A larger difference could not be obtained because of reflections in the lenses of the projectors. Intensity was adjusted with the Wratten neutral density filters and was measured by placing the thermopile detector, covered with half a transparent polystyrene vial similar to those making up the test container, on the vortex mixer (see below). Low intensities were calculated from the known optical densities of the neutral density filters.

Approximately one hundred whiteflies were dark-adapted for fifteen minutes in the test container, after which the container was mounted on a vortex mixer. All manipulation was carried out under dim red light (Kodak safelight filter No. 1A). The container was shaken vigorously on the mixer for one second in complete darkness; immediately it stopped shaking the projectors were turned on. After two minutes the training procedure commenced (see Figure 5.5). The container was shaken, with the vortex set to half speed, every five seconds for periods of two seconds during 30 seconds. The wavelength was then changed by switching one projector off and the other on. There was then a non-shaking period of 30 seconds. This procedure was repeated

Figure 5.4 Diagrammatic representation of the apparatus used to measure "fast" phototaxis and to condition the whiteflies. L, lense; I, interference filter; ND, neutral density filters; B, beam splitter. The projectors were focused on the container; slides, which were clear or blacked-out on one side were exchanged in the projectors according to the illumination desired.

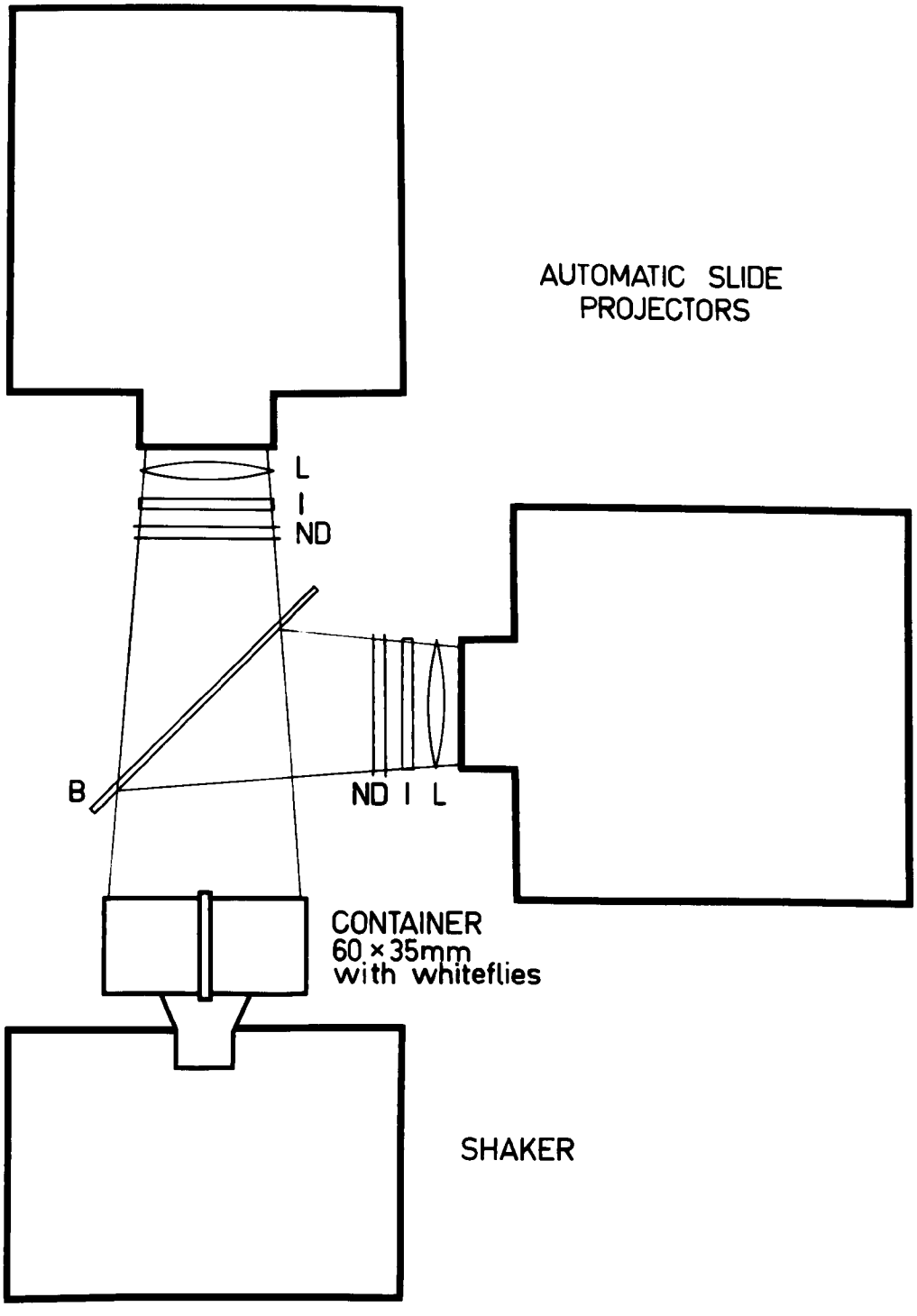
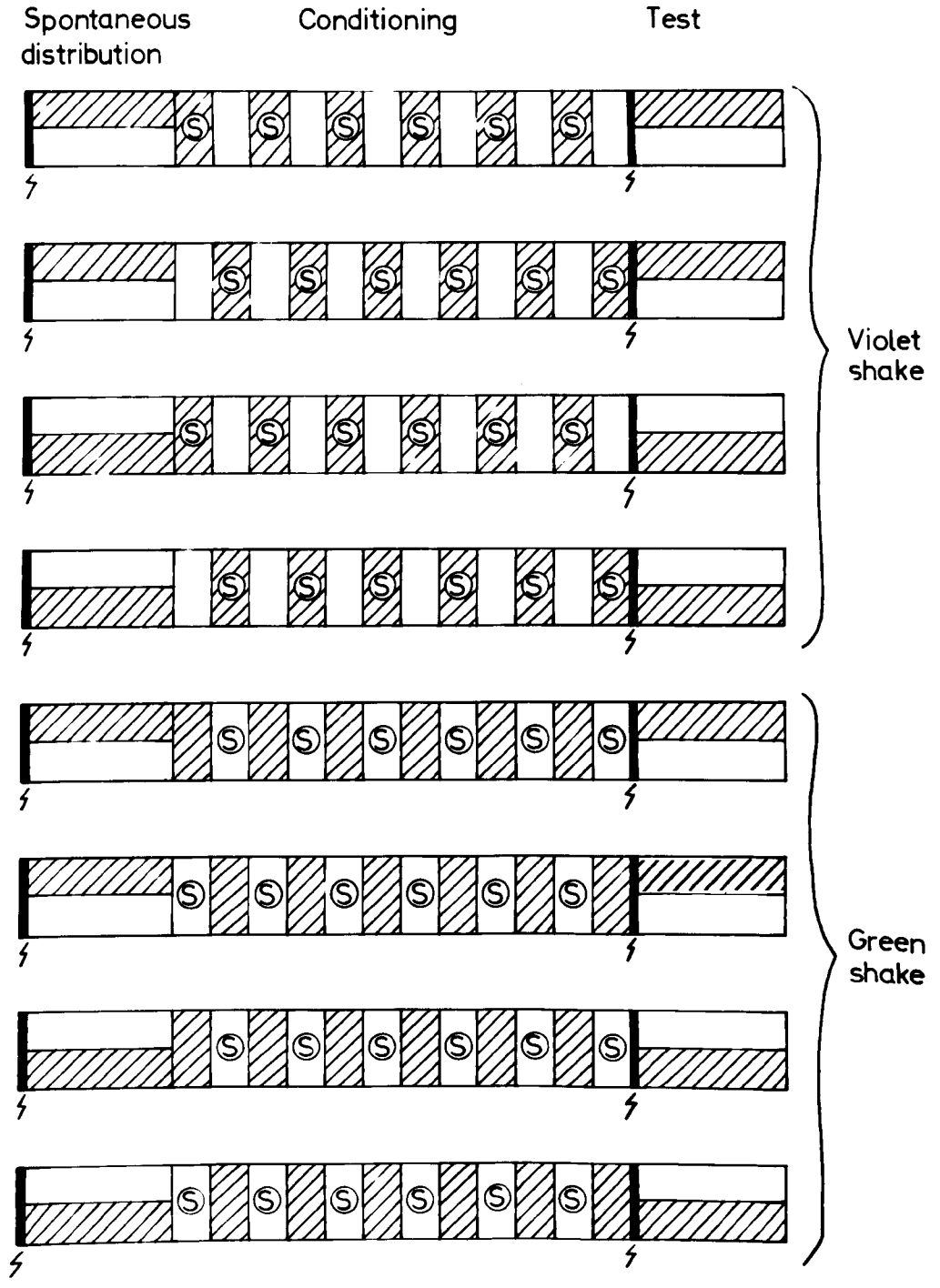

AUTOMATIC SLIDE
PROJECTORS
L
I
ND
B
ND I L
CONTAINER
60 x 35mm
with whiteflies
SHAKER

Figure 5.5 A schematic representation of the training and test procedure. Eight permutated sequences were used to ensure full symmetry with respect to order the two lights were presented, shaking and orientation of the test lights.


Spontaneous distribution
Conditioning
Test
Violet shake
Green shake
Ⓢ Shake
Shake vigorously for 1 sec.
400nm light
550nm light

six times, after which the container was shaken vigorously for one second in complete darkness in order to distribute the whiteflies evenly and then one side of the container was illuminated with the 400nm light and the other with the 550nm light for two minutes. After the two minute period, the number of whiteflies in each half of the container was counted under high intensity 550nm light. The 400nm light was turned off. Under this illumination the whiteflies moved very little and it is unlikely that it affected the final distribution of the insects.

When all the possible combinations of orientation of the lights and order of the shaking are taken into account, there are eight possible permutations (see Figure 5.5). The permutations were arranged in matched pairs, with shaking corresponding to different wavelengths within each matched pair. Each matched pair was chosen at random and the whole set of permutations was repeated four times, making up 16 replicates. The results were expressed as a percentage of the total number of whiteflies in the side of the container illuminated with 550nm. Statistical analysis was by a matched pairs t test for Experiment 1 and a randomised block design analysis of variance for Experiment 2.

5.3.1 Experiment 1

There was insufficient time to condition the whiteflies to varying intensities of the two monochromatic lights and so the choice of intensities was arbitrary. For this experiment the intensities both for conditioning and assay were set at 8×10^{13} quanta $cm^{-2} sec^{-1}$ for the 400nm light and 0.82×10^{13} quanta $cm^{-2} sec^{-1}$ for the 550nm light.

The results are shown in Table 5.3. There were significantly

Table 5.3 Conditioning Experiment 1.

Percentage of the Total in the Green (mean of 16 replicates)		
Violet shake	Green shake	
82.4 (± 2.4)	88.6 (± 1.4)	*

* - Significant at the 5% level.

more whiteflies in the green half of the container when shaking was associated with green than when it was associated with violet. This result is opposite to that which would be expected if conditioning was occurring and shaking was an aversive stimulus, as probably occurs in *Drosophila melanogaster* (Menne and Spatz, 1977; Bicker and Reichert, 1978; Reichert and Bicker, 1979). Thus either shaking is not an aversive stimulus or true conditioning did not occur. Since shaking is an unlikely (albeit possible) favourable stimulus, it would also seem unlikely that conditioning occurred. An alternative explanation of the different distribution can be related to different rates of fatiguing at different wavelengths. The whiteflies were very active when illuminated by the intensity of 400nm light used, whereas they were not active when illuminated by the 550nm light at any intensity. Thus shaking the whiteflies under 400nm illumination may have had a greater fatiguing effect than shaking under 550nm illumination.
It is unfortunate that a spontaneous distribution treatment (one second shake in darkness, then test) was not incorporated into the design of the experiment, since if true conditioning was occurring and any fatiguing effect was relatively small, the spontaneous distribution would be expected to fall between "violet shake" and "green shake". Experiment 2 was designed to eliminate any fatiguing effects that may have occurred due to differing activities.

5.3.2 Experiment 2

The aim of this experiment was to determine whether *T. vaporariorum* could be conditioned to wavelength independent of intensity, using a technique that eliminated differential activities and hence probably any differential fatiguing effects.

The conditioning paradigm was the same as Experiment 1 except that conditioning (shaking) was at a low intensity of 400nm light ($0.4x10^{13}$ quanta $cm^{-2}sec^{-1}$) and a high intensity 550nm light ($7.2x10^{13}$ quanta $cm^{-2}sec^{-1}$) while the spontaneous distribution and the test (see Figure 5.5) was a high intensity 400nm light ($8x10^{13}$ quanta $cm^{-2}sec^{-1}$) and a low intensity 550nm light ($0.082x10^{13}$ quanta $cm^{-2}sec^{-1}$). All these intensities were above the threshold of the spontaneous response (one second shake in darkness, then test) (see Figure 5.6) although it must be kept in mind that the spontaneous response is a different response from a conditioned response and therefore not directly comparable. At the low intensity of 400nm the whiteflies were not active and therefore any fatiguing effect due to differential activities should have been equal at the two wavelengths and therefore cancel out. The reversal of the intensities meant that if the whiteflies were conditioned to wavelength, rather than brightness, the conditioning index would be positive. If the conditioning effect was brightness dependent, however, the conditioning index would be negative, and if the conditioning effect was wavelength and brightness dependent, the conditioning index would be close to zero (Bicker and Reichert, 1978). This assumes that the high intensity of one wavelength appeared brighter than the low intensity of the other wavelength, but since brightness values were not estimated for the conditioning experiment, this assumption was not tested. The results from the spontaneous phototactic response (Figure 5.6) could not be used to estimate brightness values since any attempt to estimate brightness values (e.g. from intensity response functions or thresholds) should use the same behavioural response (i.e. conditioning), and spontaneous phototaxis is a different response from conditioning. However, in the absence of other data, Figure 5.6 was used as a guide. Spontaneous

Table 5.4 Conditioning Experiment 2.

Percentage of the Total in the Green (mean of 16 replicates)		
Violet Shake	Green Shake	Spontaneous Distribution
59.7 (± 1.7)	62.6 (± 1.7)	68.4 (± 1.6)
n.s.	*	

* - Significant at the 5% level.

phototactic threshold values are the same for both wavelengths (see Figure 5.6). Since at threshold the brightness functions (brightness *vs* intensity) and the intensity response functions of phototaxis probably converge, brightness values near threshold were probably about the same. The threshold intensities for phototaxis were the same for each wavelength and so equal intensity near threshold probably represented equal brightness. If deviations from univariance of the brightness functions were between zero and 1 or 2 log units (the intensity differences for conditioning Experiment 2) the above assumption may be valid. It is difficult to conceive that increasing intensity should result in decreasing brightness perception.

The results are shown in Table 5.4. There was no significant difference between the conditioned populations but both conditioned populations gave a response that was significantly less than the spontaneous distribution. This indicated that either there was no conditioning effect or any conditioning effect that may have occurred was brightness and wavelength dependent. Once again, the most likely explanation is that no conditioning took place. The lowered response of both conditioned populations compared with the spontaneous response indicated that fatiguing was occurring at both wavelengths. Thus the conditioning experiments did not produce evidence for colour vision.

It is still possible that whiteflies can be conditioned to visual stimuli (e.g. by using an improved paradigm or a conditioning stimulus other than shaking), but since development of a suitable paradigm is time-consuming, further studies on conditioning were not attempted. Another method of studying wavelength specific

behaviour and colour vision was needed. Fischbach's (1979) method of examining simultaneous colour contrast in *Drosophila melanogaster* was one such method. Fischbach's experimental design was used in the next experiments but the "fast" phototaxis paradigm used in the previous conditioning experiments was used as a behavioural measure rather than "slow" phototaxis as used by Fischbach (1979) because this method had already been used with the whiteflies and it had proved to be reliable and easy to use.

5.4 Colour Contrast

Previous experiments (Section 5.2) have shown that *T. vaporariorum* exhibits wavelength specific behaviour but did not provide any indication of the complexity of the visual system controlling this behaviour. Simultaneous colour contrast involves a change in the hue, saturation, and brightness of a test light owing to the influence of a nearby inducing colour (Graham and Brown, 1965). The presence of simultaneous or successive colour contrast effects implies a complex integration of visual inputs and may be taken as evidence for colour vision if receptor processes (such as prolonged depolarisation after illumination with certain wavelengths) can be excluded and the same behavioural responses are used (Menzel, personal communication). The following experiment was designed to test whether the system was a relatively simple wavelength specific system as postulated by Menzel (1979) or a more complex, highly developed colour vision system by testing for colour contrast effects.

The method was the same as that used in the previous section (conditioning experiments) except that the whiteflies were not conditioned, but their spontaneous distribution was measured after the projectors were turned on (i.e. shake for one second in darkness, then test).

To derive the intensity response functions (Figure 5.6) the response was recorded as a percentage of the total in the illuminated side; in the experiment involving a colour contrast (Figure 5.7), the results were recorded as a percentage of the total in the side illuminated by the standard 400nm light. Means and standard errors are given.

The wavelengths 400nm and 550nm were selected because *T. vaporariorum* clearly exhibits wavelength specific behaviour with the "settling" response at these wavelengths. *T. vaporariorum* also exhibits wavelength specific behaviour with phototaxis, since the intensity response functions for phototaxis (Figure 5.6) were different above threshold. The whiteflies were negatively phototactic to 400nm light but positively phototactic to 550nm light within the intensities used. Fischbach (1979) demonstrated simultaneous and successive colour contrast in *Drosophila melanogaster* and the same method was used to test for colour contrast effects in *T. vaporariorum*. The results (Figure 5.7) showed no colour contrast effects; in the presence of a wavelength contrast, the response was identical to the sum of the respective responses without a wavelength contrast (Figure 5.6), i.e. curve A (Figure 5.6) can be closely superimposed on curve C (Figure 5.7) by inverting and superimposing the respective responses below threshold (see Figure 5.8). This compares with "slow" phototaxis in *Drosophila*, where it is not possible to predict the response in the presence of a wavelength contrast by a linear combination of the responses to the wavelengths presented alone (Fischbach, 1979). In addition, the curves in Figure 5.7 correspond to those expected if there were no interactions of the outputs of the photoreceptors in the central nervous system. There is no shift in the threshold of curves

Figure 5.6 Intensity response functions of "fast" phototaxis for 550nm (A) and 400nm (B). The response, which was expressed as a percentage of the total number of whiteflies in the illuminated half of the container, is plotted as a function of log relative intensity where zero represents 8×10^{13} quanta $cm^{-2}sec^{-1}$. The vertical lines represent standard errors of the mean of 10 replicates.

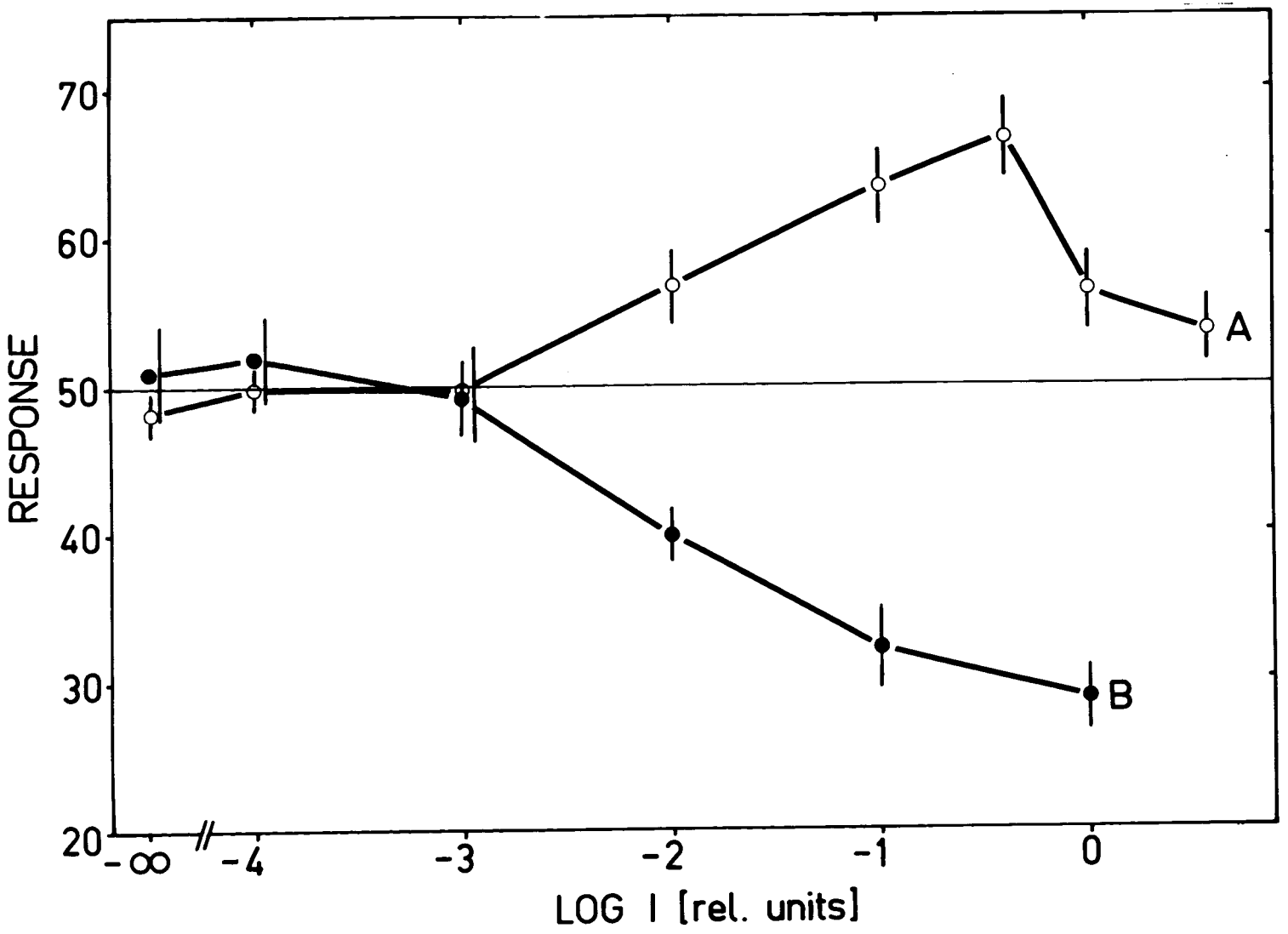

70
60
50
40
30
20
RESPONSE
-∞
-4
-3
-2
-1
0
LOG I [rel. units]
A
B

Figure 5.7 Phototaxic response of whiteflies to constant 400nm light plotted as a function of light intensity of 550nm light at the same and/or opposite side of the container. The response was expressed as a percentage of the total in the half of the container illuminated by the constant 400nm light. The intensity of the 400nm light (which is represented by zero on the intensity scale) was 8×10^{13} quanta $cm^{-2} sec^{-1}$. C, 400nm *vs* 550nm; D, 400nm + 550nm *vs* darkness; E, 400nm + 550nm *vs* 550nm; F, 550nm *vs* 550nm (control). The experiment was done in two sections ($I \leq 2.5$ and $I \geq 2.5$). The vertical lines are standard errors of the mean of ten replicates. For the sake of clarity only the largest standard errors are shown at the lower intensities.

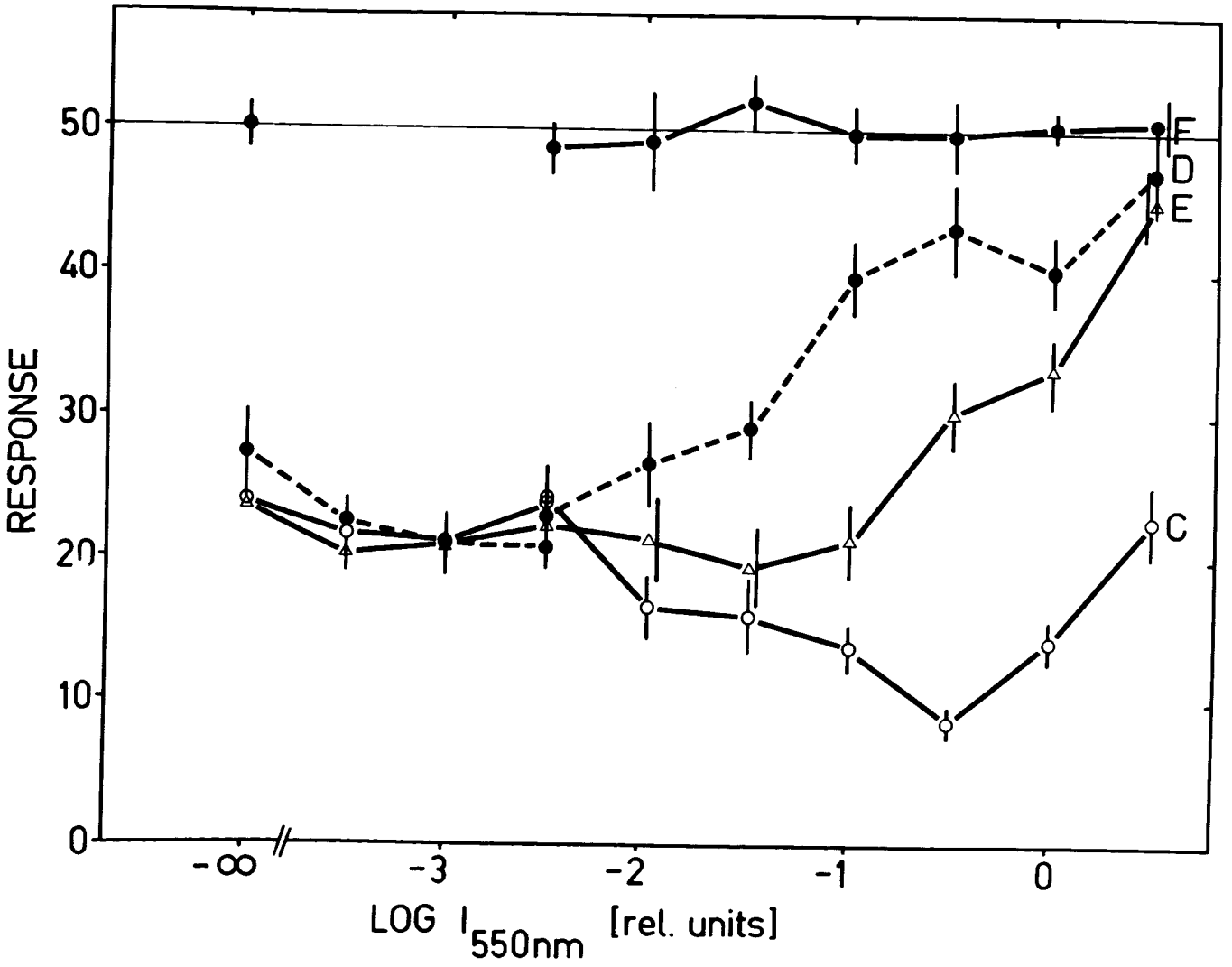
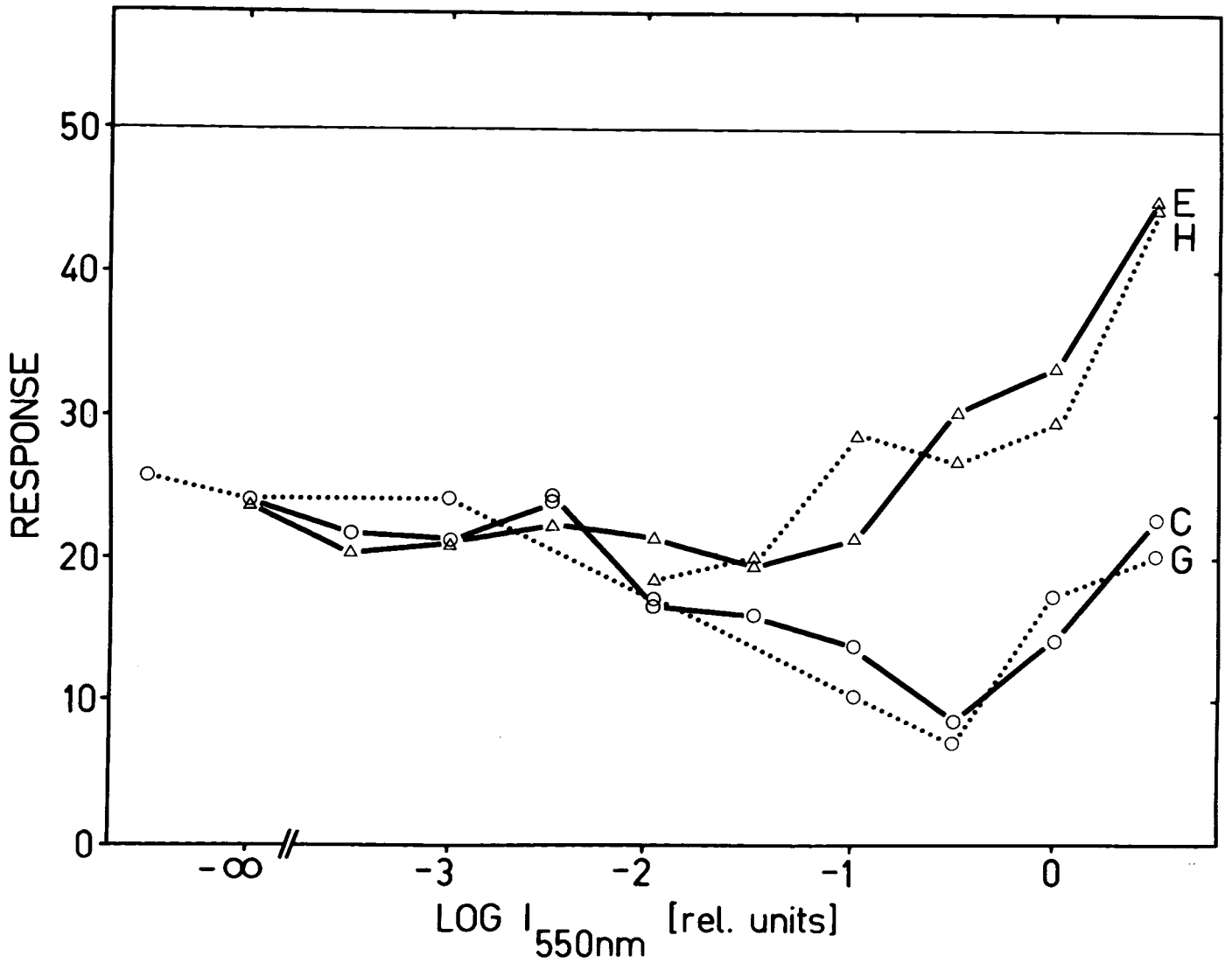

RESPONSE
50
40
30
20
10
0
-∞
-3
-2
-1
0
LOG I 550nm [rel. units]
F
D
E
C

Figure 5.8 C, as in Figure 5.7; G, curve A (Figure 5.6) superimposed on C by inverting (because of the way A is plotted) and superimposing the responses below threshold; E, as in Figure 5.7; H, C + D (Figure 5.7) minus the response to 400nm presented alone. Intensities and response as in Figure 5.7


RESPONSE
50
40
30
20
10
0
-∞
-3
-2
-1
0
LOG I 550nm [rel. units]
E
H
C
G

D or C (Figure 5.7) which differs from Fischbach's (1979) results, where he found a shift in threshold of the equivalent of curve D; but there is a shift in the threshold of curve E, which is as expected if there were no interactions of the outputs of the photoreceptors. The curves are apparently additive, as Fischbach (1979) found, i.e. C + D minus the response to 400nm alone = E (Figure 5.8).

5.5 Discussion

The intensity response functions (Figure 5.6) show that at least two photoreceptors with different spectral sensitivities are involved in the phototactic response because the response is not univariant, but there are no colour contrast effects or interactions of the outputs of photoreceptor types in the central nervous system for these two wavelengths. Therefore there is no evidence for colour vision in the phototactic paradigm.

Although the outputs of the different photoreceptors do not interact in the phototactic paradigm, they may interact in other behavioural responses such as food-plant selection, "slow" phototaxis, the optomotor response, or the "fall reflex" observed by Moericke *et al.* (1966). Fischbach (1979) tested "slow" phototactic behaviour in *Drosophila melanogaster* and it is possible that the difference between his results and those reported here may have been due to the different behavioural paradigms used rather than the different animals. The "settling" response measured a response analogous to food-plant selection, namely, landing after a short flight, and is also related to the length of time spent on each surface after a landing. It is wavelength specific and may or may not involve colour vision. One other factor that must be considered is that the result may have been dependent on the wavelengths used or the design of the test arena,

although it is doubtful that other wavelength paris would have produced evidence of interactions of photoreceptor outputs since the wavelengths used (400nm and 550nm) were chosen carefully, based not only on the results from the "settling" response paradigm but also on a number of preliminary trials using other wavelength pairs. Colour contrast effects are dependent on the relative sizes of inducing and test fields, proximity of the two fields and relative brightness (Graham and Brown, 1965), any one of which may not have been optimised in the "slow" phototactic paradigm.

Moericke *et al.* (1966) found that the "fall reflex" of *Trialeurodes vaporariorum* was apparently intensity independent and this has been taken as evidence of "colour processing" by Schümperli (1973). However, although Moericke *et al.* showed that the "fall reflex" occurred to yellow and not to any of a series of grey papers ranging from white to black, this does not mean that *T. vaporariorum* has true colour vision. This type of behaviour can occur when there is no interaction of photoreceptor outputs in the central nervous system. If, for example, there were two non-interacting photoreceptors, one controlling one behavioural pattern and the other controlling a different antagonistic behavioural pattern (e.g. if yellow stimulated landing and inhibited flight while ultra-violet inhibited landing and stimulated flight) the resultant behaviour would be apparently intensity-independent because the behaviour would depend on the relative outputs of the photoreceptors and hence the absolute spectral distribution of the stimulus. This may have occurred in the phototactic paradigm of *T. vaporariorum*. The opposite phototactic responses to 550nm and 400nm (positive to 550nm, negative to 400nm) and the shapes of the curves in

Figure 5.7 support this supposition. If there were no interactions at all, both at the photoreceptor and also the behavioural level, one would expect curve D (Figure 5.7) to be a mirror image of curve C, and curve E to remain at threshold (= response to 400nm alone). However what is observed is that curve D is the mirror image of curve C below a certain critical intensity, above which curve D probably tends towards curve A (Figure 5.6) and curve E (Figure 5.7) remains at threshold until this critical intensity is reached, above which it probably tends towards curve F. The fact that the curves behave as expected close to threshold (unlike *Drosophila* (Fischbach, 1979)), coupled with the fact that there are no colour contrast effects, indicates that there are probably no interactions of photoreceptor outputs in the central nervous system. However, because the curves deviate from expectation at higher intensities, and in fact appear as if the behavioural pattern controlled by the 550nm light is increasingly inhibiting the behavioural pattern controlled by the 400nm light as intensity increases, the two behavioural patterns are probably antagonistic. This supports Menzel's (1979) hypothesis that chromaticity-coding systems early in evolution may have been antagonistically organised between behavioural patterns sensitive to short- and long-wavelengths. However, interaction between receptors at high intensities cannot be completely ruled out.

Although *Drosophila* shows simultaneous and successive colour contrast effects in its "slow" phototactic behaviour (Fischbach, 1979), this does not necessarily mean that colour vision in flies is as highly developed as in ants, bees or humans and may not even fit the concept of "true" colour vision, especially since Bicker and Reichert (1978)

have shown that conditioning in *Drosophila* is wavelength- and intensity-dependent. Even so, their conditioning paradigm did not allow them to condition the flies to wavelength differences only. The results reported here, when compared with *Drosophila*, show the necessity to differentiate between wavelength specific behaviour without interactions of photoreceptor outputs (*T. vaporariorum*) and wavelength specific behaviour with interactions of photoreceptor outputs (*Drosophila melanogaster*) and are to date the best established case of wavelength specific behaviour without interactions between input channels. Such results also support Fischbach's (personal communication) concept of an evolutionary continuum towards "true" colour vision, ranging from wavelength specific behaviour with no interactions between input channels to the central nervous system (i.e. photoreceptor outputs), through to variously highly developed "true" colour vision systems.

CHAPTER 6

6. GENERAL DISCUSSION

Selection of food plants involves a chain of responses beginning with orientation to the plant from a distance by olfactory or visual stimuli. The use of visual stimuli is likely to be non-specific, i.e. it will tend to cause the insect to land on any surface that reflects or emits light of the appropriate wavelengths (usually a green plant); thereafter, other stimuli such as gustatory and tactile as well as olfactory and visual stimuli may signal whether the insect has landed on a suitable food-plant. There is only one example of selective visual behaviour, that of *Hyalopterus pruni* (Moericke, 1969). This insect is attracted to coloured plates in the range of orange to yellow-green with an optimum at yellow but only when these colours are unsaturated. This is probably an adaption to the unsaturated colour of its food-plant. It is likely that *Trialeurodes vaporariorum* uses visual stimuli to guide it to a green plant, since initial landings of whiteflies appear to be unrelated to the suitability of a food-plant but are strongly related to colour (Vaishampayan, 1975a, b; Verschoor-van der Poel and van Lentern, 1978). However, the results of previous studies are difficult to interpret because light intensities were not sufficiently controlled and the role of ultra-violet light was neglected. The present project aimed to overcome these difficulties and to examine various aspects of visual behaviour, and in particular, wavelength specific behaviour and colour vision. Aspects of the visual behaviour which have been examined are "slow" phototaxis, flight behaviour, walking and take-off behaviour, the "settling" response, the "fall reflex" and "fast" phototaxis.

A spectral efficiency function for "slow" phototaxis (see Chapter 3) showed a peak at 400nm. This result was different from MacDowall's (1972) spectral efficiency function measured using a response similar to the "settling" response, which peaked at 550nm. However, the observed difference between MacDowall's (1972) results and those obtained using "slow" phototaxis, can be explained by the supposition that MacDowall measured a different behavioural response which had a different spectral sensitivity. Studies on walking behaviour, flight and landing behaviour have elucidated this. Whiteflies, when in flight, oriented towards 400nm compared with 550nm, walked faster towards 400nm, and took off more readily when illuminated by 400nm light. The "fall reflex", which is probably a prelude to a landing response, occurred in response to 550nm monochromatic light, but not to 400nm. Since "slow" phototactic behaviour mainly consists of walking, the whiteflies walked faster towards the shorter wavelengths, but because MacDowall's (1972) method involved flight, landing, and take-off behaviour, his spectral efficiency function showed a maximum at 550nm, because once the whiteflies landed on a surface illuminated by a 550nm light (landing is probably stimulated by 550nm light), they tended to stay ("settled") (see Section 4.8), while if they landed on a surface illuminated by shorter wavelengths, the probability of take-off was greater (see Section 4.8). The end result of this interaction of behavioural responses can be illustrated by the "settling" response (see Section 5.1) which was similar to MacDowall's (1972) method and gave similar results. Thus the whiteflies used here behaved similarly to MacDowall's whiteflies and also showed the "fall reflex" in response to 550nm monochromatic light (see Section 4.9), which confirmed

Moericke *et al.*'s (1966) results. These similarities make it unlikely that a change in this visual behaviour could explain the recent increase in the whitefly population in Australia.

The "settling" response was deliberately designed to simulate a population of whiteflies landing on a food-plant after a short flight, using visual cues only. "Settling" was not used in the same context as in Kennedy's flight chamber experiments with aphids, in which "settling" involved, (1) cessation of wing beating, (2) and (3), probing and walking alternatively (4) going under a leaf (5) feeding and (6) larviposition (see Kennedy and Booth, 1963b). For the purposes of this study, "settling" involved alighting and remaining on a surface for a short period, rather than landing and taking off immediately. Flight chamber experiments would probably have yielded more detailed information on flight and settling behaviour and the results may have been more easily applied to the field situation, but attempts to use a flight chamber failed. Nevertheless, whiteflies accumulated on the surface illuminated with green monochromatic light of 550nm (see Sections 4.6.2 and 5.2), and again this observation supports the hypothesis that *T. vaporariorum* may find its food-plant by visual cues. This behaviour is wavelength specific and, in the absence of a specific test, may or may not involve colour vision.

Because of insufficient time, flight, landing, take-off and walking behaviour were examined at only one quantum flux level and so the conclusions of these experiments (Chapter 4) can only be applied at this particular intensity. It would be useful to know the action spectrum of each behavioural pattern, and the spectral sensitivities

of the receptor cells in various parts of the eye, since this would help to elucidate the contribution of the receptors to various behavioural patterns. This would require a far more detailed analysis of the various behaviour patterns at varying intensities and wavelengths. One other approach has been taken with *Drosophila*, where various receptor-degenerate mutants have been developed (reviewed by Heisenburg, 1979), but this method is probably only feasible with *Drosophila* because of the large amount of genetic background information available.

Conditioning experiments did not produce evidence for colour vision, but it is likely that the whiteflies were not successfully conditioned. Shaking was used as an aversive conditioning stimulus but it probably had differential fatiguing effects on the whiteflies at the wavelengths used (400nm and 550nm), because the insects were active under 400nm illumination whereas they were not active under 550nm illumination. It may still be possible to condition whiteflies to visual stimuli, for example, by using wavelengths under which the whiteflies are equally active, or by using a completely different conditioning stimulus (other than shaking). A conditioning paradigm using stimuli important to the survival and reproduction of the whiteflies is most likely to be successful. Members of a species have certain well-defined requirements for individual learning; for example, learning to recognise a mate, young or territory, or in acquiring skills necessary for food-finding or nest building (Shettleworth, 1972). It would seem likely that members of a given species would learn adaptive behaviour more efficiently than other kinds of behaviour (Shettleworth, 1972). Thus a learning paradigm for whiteflies would probably be most successful

if it was based on food-plant selection, for example, since finding a suitable food-plant is one of the most important factors determining whether a whitefly survives and reproduces. However, this does not mean that whiteflies cannot be conditioned by shaking. Whiteflies are subjected to shaking in the field when the wind blows the leaves of its food-plant. The conditioning experiments (Section 5.2) may not have produced the right kind of shaking (frequency and amplitude) or the length of time shaken or the number of reinforcements may not have been optimised for maximum conditioning effect. Since whiteflies are subjected to shaking on a food-plant, it is possible that shaking might be a favourable stimulus rather than an aversive stimulus, but at this state it is uncertain as to whether shaking is aversive or favourable. Development of a suitable learning paradigm for *T. vaporariorum* may be difficult, because of the difficulty of feeding them in circumstances suitable for an appropriate experiment, but an attempt would probably be worthwhile because training techniques are powerful methods of studying visual behaviour and the mechanisms underlying this behaviour.

The shaking technique was used to elicit the "fast" phototactic response described in Section 5.3. This response was wavelength specific and at least two spectral types of photoreceptors contributed to the response. The lack of any colour contrast effects or evidence of interactions of input channels into the central nervous system indicated that in "fast" phototaxis the visual system probably does not code "colour". It is possible that the central nervous system may weight the inputs from the receptors differently for different

behavioural responses and some interactions may occur in this type of system, but the main conclusion to come from the experiments in Section 5.4 (colour contrast experiment) is that the neuronal complexity of wavelength specific behaviour of "fast" phototactic behaviour in whiteflies is likely to be simpler than "true" colour vision as it occurs in ants and bees (and probably also *Drosophila*). The complexity can further be elucidated if the spectral sensitivities of the receptors was known as well as the spectral sensitivities of the various behavioural patterns. If the spectral sensitivities of the receptors matched the sensitivities of the behaviour patterns then this would be further evidence for "true" wavelength specific behaviour. This is precisely what occurs in dance orientation of bees (see Edrich *et al.*, 1979; Edrich, 1979). In bees dancing on a horizontal comb, the direction of the sun is inferred from light perceived by the 450nm and 550nm receptors and not by the 350nm system. The 350nm system, when stimulated with unpolarised ultra-violet light, causes a different type of orientation - "anti sun orientation" (Edrich *et al.*, 1979). Polarised light is perceived only by the ultra-violet receptors (von Helversen and Edrich, 1974). Ultra-violet orientation is qualitatively distinct from sun orientation (Edrich, 1979); the ultra-violet and visible receptors control different behavioural patterns. Thus orientation is wavelength specific, only large chromatic differences are important. In comparison, behaviour related to food gathering can be shown to be much more sensitive to chromatic differences. Bees can be trained to distinguish between wavelengths differing by as little as 4.6nm (von Helversen, 1972). Thus demonstration of a lack of colour contrast effects, and the likelihood of no interactions

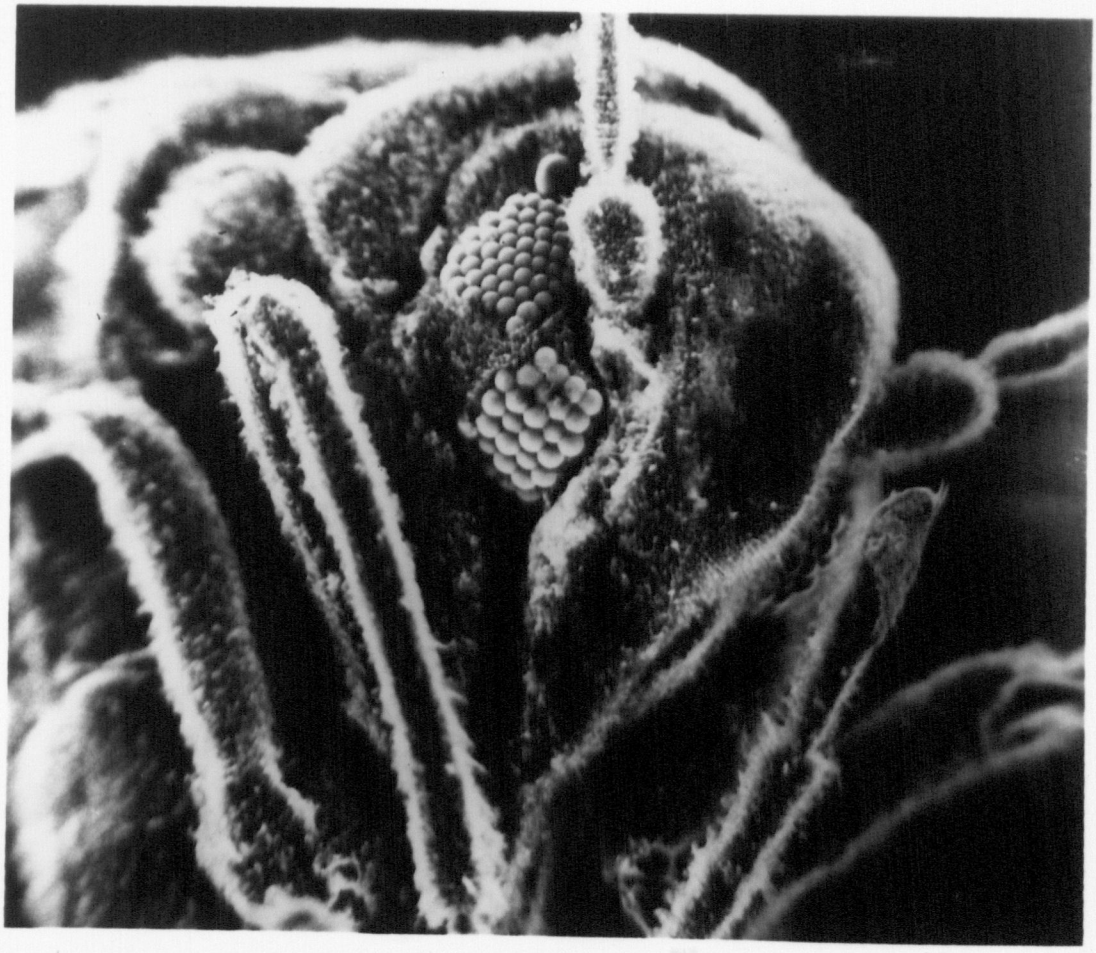
of photoreceptor outputs in the central nervous system in "fast" phototaxis, is by no means conclusive evidence against the existence of colour vision in *T. vaporariorum*, although some preliminary observations of wavelength mixtures in "slow" phototaxis and the "settling" response, suggested results with these responses would be similar to those obtained with "fast" phototaxis.

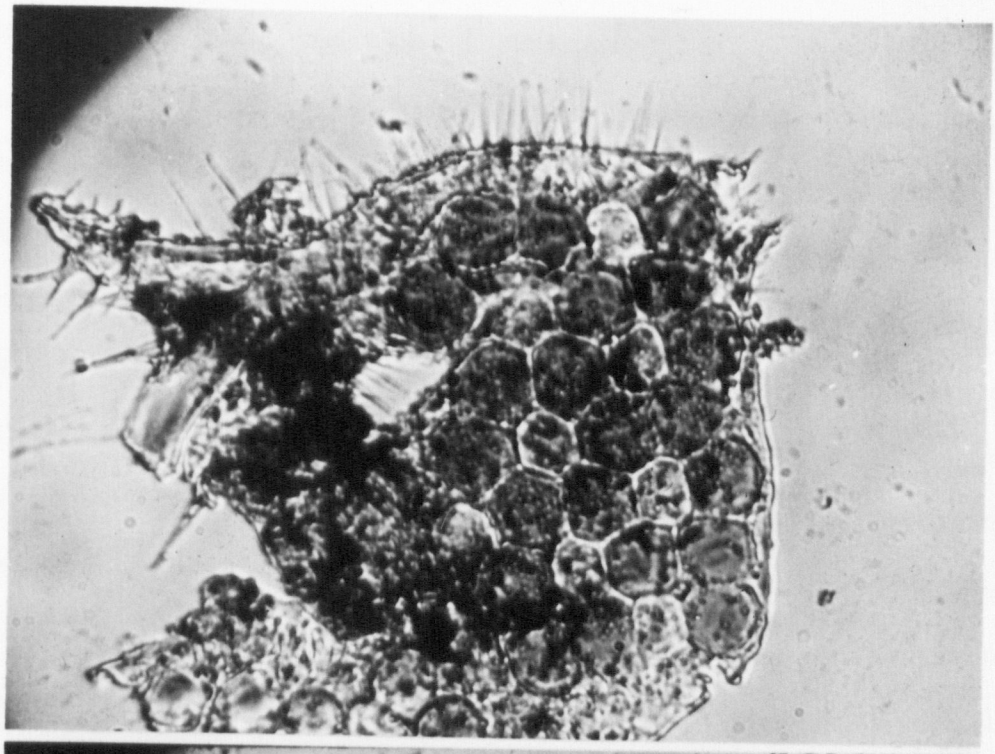
The compound eye of *T. vaporariorum* consists of two halves, a ventral half of 30 ommatidia and a dorsal half of approximately 50 ommatidia (see Plate 4). As in *Aleyrodes brassicae* (Eltringham, 1931), *T. vaporariorum* also has yellow corneal filters in the ventral half of the compound eye (Plate 5) but not in the dorsal half (Plate 6). These corneal filters are arranged in a hexagonal pattern with a clear facet in the centre surrounded by six yellow facets.

Bernard (1971) has suggested that corneal filters may be involved in colour vision, and it is possible for an insect with only one type of photoreceptor to have true colour vision if it also has corneal filters, since a receptor cell in an ommatidium without a corneal filter would have a different sensitivity from one with a filter with a different spectral transmittance. Thus a single spectral type of photoreceptor with different corneal filters in different ommatidia could conceivably function in a way similar to two different photoreceptors having differing spectral sensitivities. It has also been suggested that corneal filters may enhance colour contrast (Bernard and Miller, 1968; Bernard, 1971).

Kong *et al.* (1980) have shown that the grasshopper *Phlaeoba*,

Plate 4 Scanning electron micrograph of a whitefly showing the divided compound eye. (x 350)





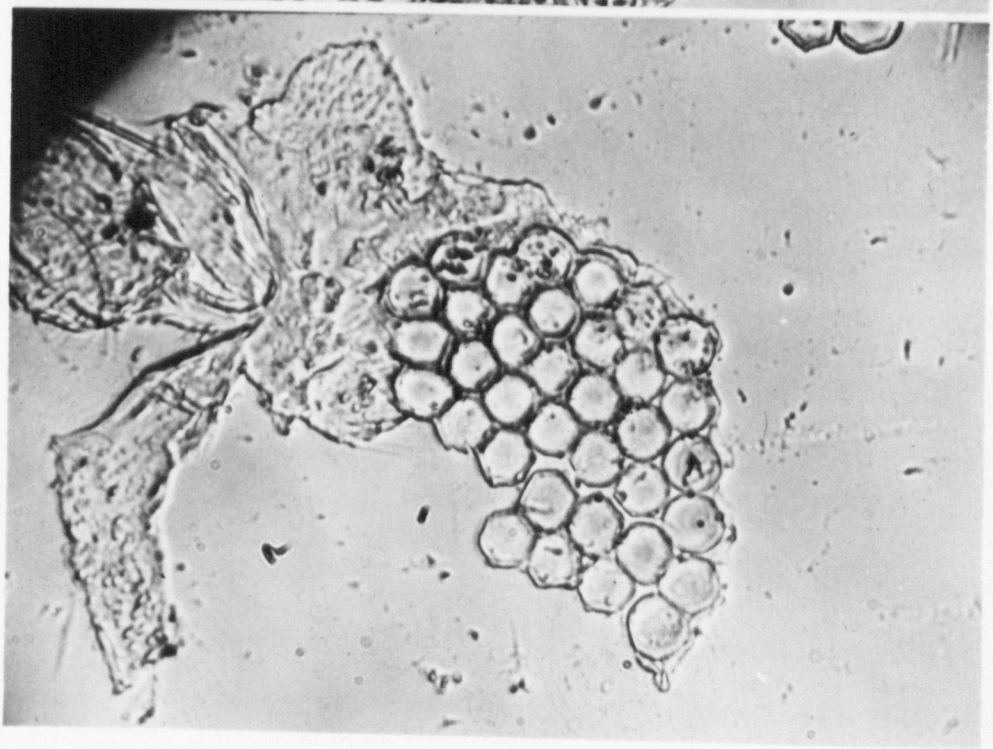


Plate 5 Cornea dissected from the ventral half of the compound eye showing the yellow corneal filters. (× 800)

Plate 6 Cornea dissected from the dorsal half of the compound eye. (× 800)

which has alternating bands across the compound eye which appear brown or clear due to screening pigments, probably has only one visual pigment (i.e. one type of photoreceptor). They have also shown that *Phlaeoba* probably has true colour vision. This supposition is based on evidence from electroretinograms (ERG) and behavioural studies. ERGs have different action spectra in the different bands and spectrally selective whole-eye adaption to light of either long or short wavelengths, yielded identical action spectra. This suggests that the eye has only one visual pigment whose spectral sensitivity is altered in the brown bands by a screening pigment. Such screening pigments are known to alter the action spectra of other insect and invertebrate eyes (Kong and Goldsmith, 1977; Goldsmith and Bernard, 1974). In spontaneous colour preference tests (measured behaviourally), the grasshoppers preferred green at the equal "brightness" level (determined from the ERG). When some red was mixed with the green light the preference for the mixture was less than for the green alone, even though the intensity of the mixture was greater. However, an ERG is not a good measure of "brightness" for behavioural tests. If, for example, an animal has two different photoreceptors of differing spectral sensitivities (or one kind of photoreceptor with and without a corneal filter), each receptor system controlling a different behavioural response, then "brightness" values would be different for each behavioural pattern and may bear no simple relation to the ERG. Thus equal "brightness" must be estimated using the same behavioural response used to test for colour vision. Kong *et al.*'s (1980) other behavioural experiments were also inconclusive. Although they presented an intensity response function for green *vs* red (green varying in intensity, red constant), they did not present red *vs* red or red *vs* green (red

varying in intensity). Considering the experiment in which they mixed varying intensities of red with green (*vs* green or red) and observed a reduction in the response to the mixture (even though the mixture was more intense) one could only conclude that the grasshoppers had true colour vision (response is non-additive) if the slope of the intensity response functions of red *vs* red or red *vs* green (red varying in intensity) were positive. This assumption was not tested. If these functions had a negative slope, Kong *et al.*'s (1980) results could be interpreted only as wavelength specific behaviour. Although Kong *et al.* showed that the grasshoppers responded more strongly to more intense white light, this does not necessarily mean that they would respond more strongly to more intense red (compared with less intense red). Thus Kong *et al.*'s results were inconclusive as to whether *Plaeoba* shows wavelength specific behaviour or true colour vision, but they did show that screening pigments can alter the spectral sensitivity of a single photoreceptor, enabling the animal to show wavelength specific effects in its behaviour, and presumably corneal filters can cause similar effects.

The function of the corneal filters in *T. vaporariorum* is unclear. Although a number of hypotheses can be formulated, they remain as yet untested. It is possible that the filters may be involved in a colour vision system as postulated by Bernard (1971), or they may enhance colour contrast. To test this hypothesis, a behavioural response which involves the lower half of the eye only, such as the "fall reflex" or a landing response, would need to be formulated. The "fall reflex" is probably unsuitable, however, because of the difficulty of mounting such a small insect and the low proportion of whiteflies which exhibited this behaviour (see Section 4.9). The "settling" response is also probably unsuitable because this behavioural response involved illumination of the dorsal as well as the ventral halves of the compound eye. "Fast"

phototaxis (Section 5.3) has already been shown not to involve a colour vision system, but it is possible that other responses may give different results.

It is possible that the ommatidia with the corneal filters specifically trigger or control a particular behavioural pattern while the other ommatidia in the ventral half of the eye control a different behaviour pattern. The dorsal half of the eye could have a different function altogether. It is unlikely that the corneal filters are involved in a true colour vision system because (1) "fast" phototaxis is wavelength specific only (both halves of the eye were illuminated) and (2) the filters are yellow. *T. vaporariorum* responds strongly to yellow in spontaneous colour choice experiments (Lloyd, 1922; MacDowall, 1972; Vaishampayan, 1975a) and so the maximum response of this behavioural pattern corresponds to the colour of the corneal filters. Since maximal wavelength discriminative ability falls between the maximum sensitivities of photoreceptors involved in a true colour vision system, one would expect the maximal sensitivity of the photoreceptors to be on both sides of the spectrum of the maximal behavioural response if that response is wavelength discriminative. A filter whose spectral transmittance corresponds to the maximal behavioural response would not enhance colour discrimination but would increase the brightness of objects reflecting those particular wavelengths. Thus the corneal filters probably function by increasing the brightness of green plants relative to other background colours, but in the absence of spectral transmittance data of the corneal filters and spectral sensitivities of the receptor cells, this view is rather speculative. Dorsal/ventral divisions of spectral sensitivity are not uncommon in insect eyes (Menzel, 1975; Laughlin and McGinnes, 1978)

and similar chromatic divisions also occur with coloured oil droplets of birds (Rodieck, 1973). The function of these oil droplets is unclear, although it may be related to chromatic aberration (Bowmaker, 1980).

Spectral transmittance data of the corneal filters and data on spectral sensitivities of the receptor cells would be useful in elucidating the function of the filters and would be especially interesting, because to date no spectral sensitivity work has been done on insects with corneal filters (Menzel, 1979; Miller, 1979).

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