J. Exp. Biol. (1970), 53, 221–229
With 8 text-figures
Printed in Great Britain

THE VISUAL MECHANISMS OF *TENEBRIO MOLITOR*: SOME ASPECTS OF THE SPECTRAL RESPONSE

BY U. YINON*

The Vision Research Laboratory, Hadassah University Hospital, Jerusalem, Israel

(*Received 2 April* 1970)

INTRODUCTION

Granaries and food storage depots provide an essentially dark environment offering only occasional low-level illumination which is rarely intense enough to make spectral discrimination possible. Nevertheless insects adapted to such environments have been found to display phototactic responses (Balfour & Carmichael, 1928; Rahman & Sohi, 1939; Perttunen & Lahermaa, 1963; Yinon & Shulov, 1966; Sohi, 1966; Soderstrom, 1968).

The low-light habitat of 'stored product' and 'granary' insects seemed to provide an environment which would demand a less well-developed spectrally sensitive visual mechanism than that found for example in field insects.

The yellow mealworm beetle *Tenebrio molitor* was chosen as a suitable 'stored product' insect to test the above hypothesis.

The spectral response of 'stored product' and 'granary' insects has been examined behaviourally by Stermer (1959), Sohi (1966) and Yinon & Shulov (1966). In that these insects display a negative phototactic response there seem to be inherent difficulties in quantifying behaviour. Further, owing to the influence of extraneous social factors the behavioural criteria themselves appear to be somewhat equivocal. To circumvent these problems the response of the mediating sense organ (the compound eye) was directly investigated by means of electrophysiological recordings of the insect electroretinogram (ERG).

METHODS

The insects used were the normal (wild type) and pale-eyed mutant adults of the yellow mealworm beetles *T. molitor* L. (Coleoptera: Tenebrionidae). The pale-eyed mutants lack the screening pigment of the typical compound eye (Ferwerda, 1928). Although visual screening pigments are not directly implicated in the mechanism of colour vision, it is of importance to consider the possible effect of these pigments upon the recording of accurate spectral sensitivity curves (Burkhardt, 1964). For this reason, seven mutants were included among the 27 experimental animals.

The specimens included both sexes, the determination of sex being made in accordance with Tuxen (1956).

Dark adaptation was provided before and during the tests according to the following

* Present address: Department of Ophthalmology, Duke University Medical Centre, Durham, North Carolina 27706, U.S.A.

schedule: 5–7 min. of dark adaptation before each testing series; 3–5 min. between stimuli group at different wavelengths; 1–3 min. between stimuli group at different intensities; and a 1 sec. dark interval between stimuli at each intensity. The starting wavelength (short, medium or long) was not found to influence the results.

The techniques have been previously described (Yinon & Auerbach, 1969). Zirconium and mercury lamps in conjunction with a monochromator provided the light sources for the optical system. A computer of average transients was used to increase the signal to noise ratio, thus allowing the recording and display of low-intensity responses, e.g. those at threshold intensities. The number of averaged responses ranged between 20 and 200, depending upon the clarity of display. The size of the averaged sample seemed to have no effect upon the form of the derived sensitivity curve even if 700 responses were averaged.

The spectral sensitivity of the principal negative potential was calculated as the reciprocal of the log of the relative intensity needed to elicit amplitudes of equal size in each wavelength. The spectral efficiency curves are based on the response height at the various wavelengths in equal-energy condition.

Appropriate statistical analyses were performed (Snedecor, 1964).

RESULTS

The ERG waveform of *T. molitor* derived from brief stimuli of continuous spectrum (tungsten lamp) has been previously analysed (Yinon, 1970). The waveform is characterized by a sharp negative potential followed by a small gradual positive potential. This general pattern is constant across the spectrum (Fig. 1). Variations in the pattern of the ERG are found in a high-frequency, low-amplitude component variously superimposed upon the general pattern. The two major ERG components as well as the high-frequency components yield analogous spectral efficiency curves thus indicating their derivation from similar receptor types.

The visible spectral range of *T. molitor* lies between 350 mμ or less and 700 mμ (Figs. 2, 3). The sensitivity at wavelengths below 350 mμ was not investigated because of technical limitations; but insects may be sensitive to shorter wavelengths (Walther & Dodt, 1959).

As the stimuli range across the spectrum the spectral efficiency and the spectral sensitivity curves of the negative potential component seem to vary systematically. As the stimuli proceed in the long-wavelength direction from the point of maximum sensitivity a sharp and continuous decrease in the amplitude, especially prominent in the sensitivity curves, may be observed. Proceeding in the short-wavelength direction the decrease in sensitivity is more gradual with a minimum at 400 mμ and a gradual increase in sensitivity to 350 mμ. The limit to the spectral sensitivity at long-wavelengths fell between 640 and 700 mμ for various specimens (Figs. 2, 3). In the short-wavelength region the response at 350 mμ was *c.* 25% greater than that found at the long-wavelength limit.

The spectral efficiency curves display a point of maximum sensitivity between 520 and 540 mμ; the maximum of the spectral sensitivity curves lies between 530 and 550 mμ.

In additional experiments which were carried out specifically in order to locate the

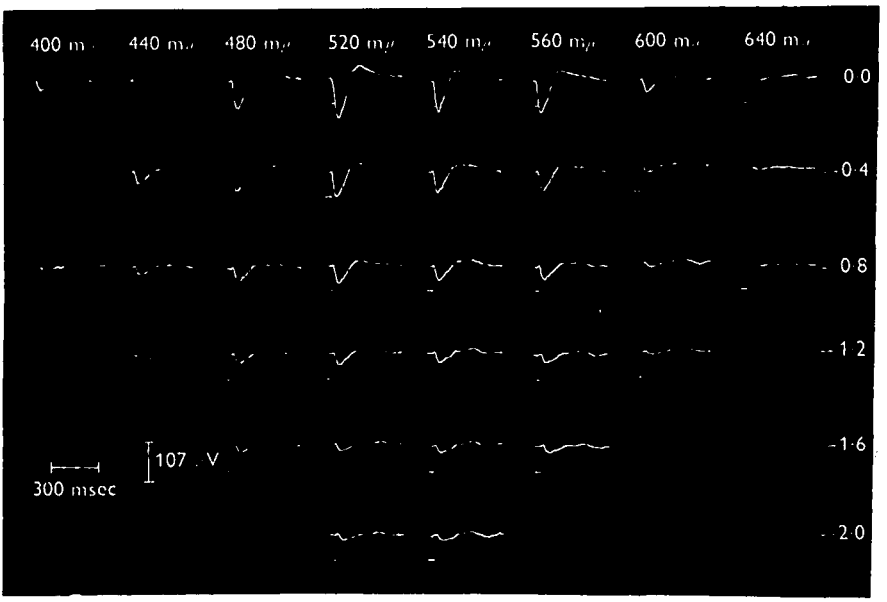

Fig. 1. Changes of the ERG at various wavelengths and light intensities. Trace under each impulse represents the stimulus duration. Ten impulses were averaged for every ERG.

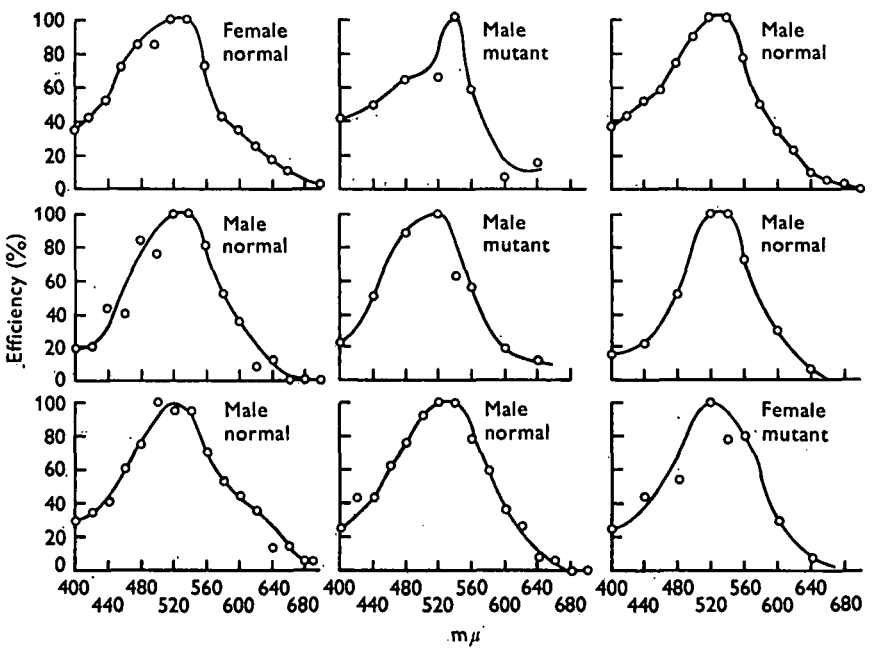

Fig. 2. Spectral efficiency in the visible spectrum (negative potential); each curve represents response of a single specimen. T-test between responses of normal and mutant animals indicating a non-significant difference ($P > 0{\cdot}05$) between the positions of the peaks. The T-test has been also applied to the data of Fig. 3 when calculated as spectral efficiency.

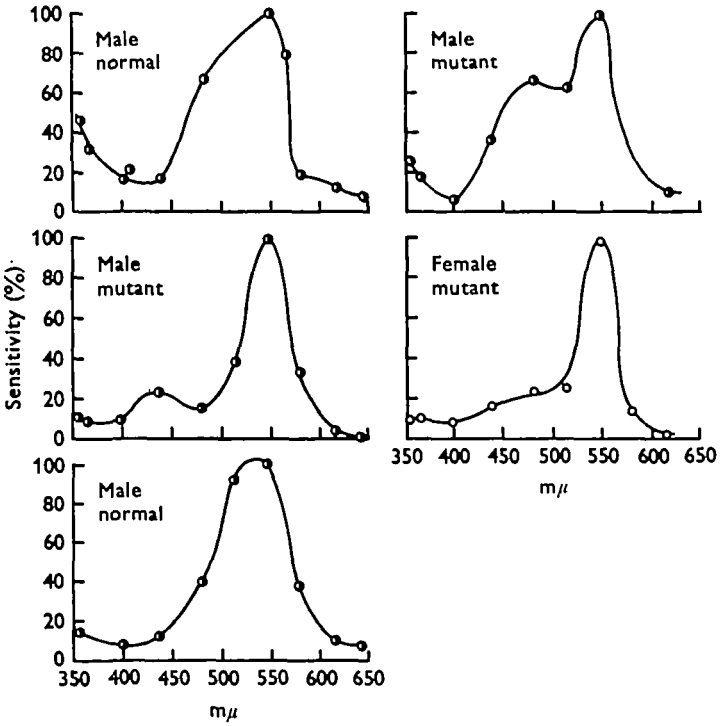

Fig. 3. Spectral sensitivity curves of single specimens in the visible and in the ultraviolet spectra (negative potential). (For statistical analysis of responses between normal and mutant animals, see legend to Fig. 2.)

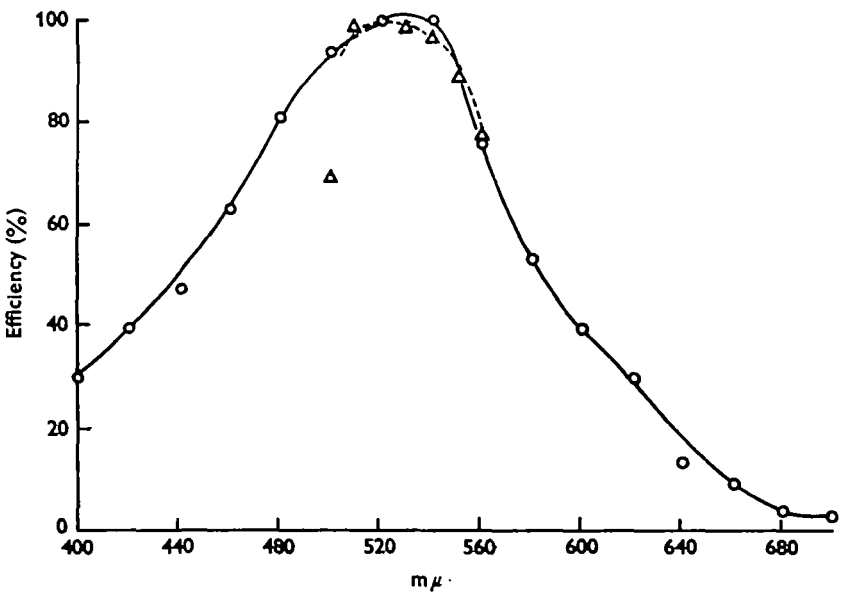

Fig. 4. The spectral efficiency (negative potential) when tested at intervals of 20 mμ ○—○ (5 specimens) and of 10 mμ at the peak region △----△ (8 specimens). Analysis of variance (F-test) between response averages in wavelengths at intervals of 10 mμ show a non-significant ($P > 0{\cdot}05$) differences in the range of 510–550 mμ.

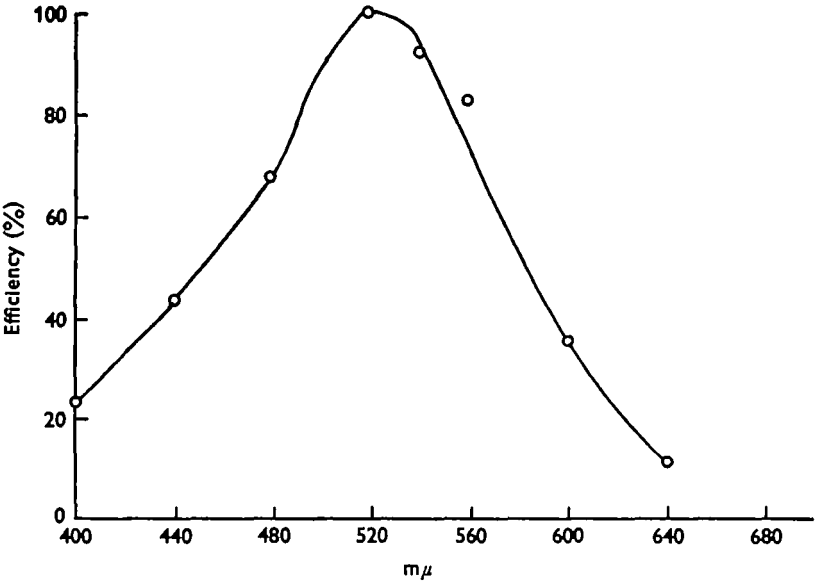

Fig. 5. Spectral efficiency of the positive potential (three specimens).

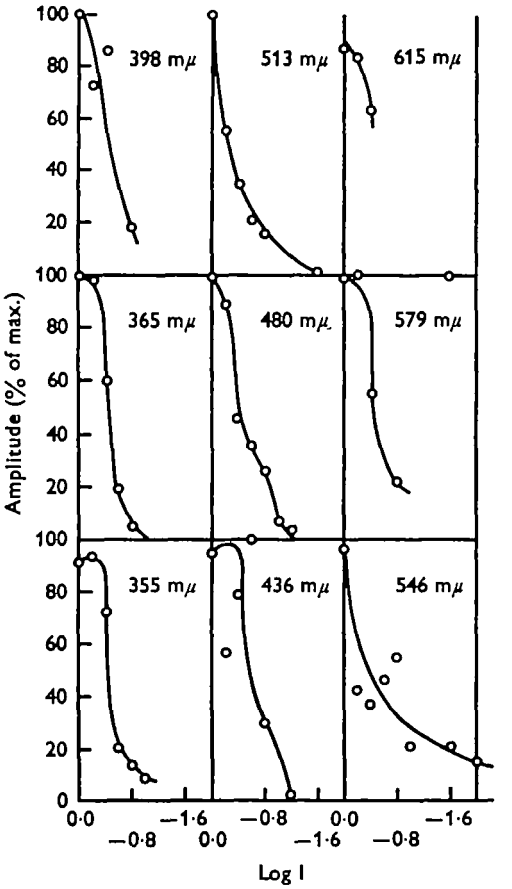

Fig. 6. Changes of the positive potential at various wavelengths as function of light intensity (five specimens).

peak more exactly, the response was tested in intervals of 10 mμ between 500 and 560 mμ. It was found that the difference between the averaged responses in the range of 510–550 mμ was not significant ($P > 0{\cdot}05$) (Fig. 4). The peak and the other components of the spectral efficiency curve were the same. Varying the stimulus intensities resulted only in changes in the response level. This finding agrees with that of Stermer (1959) but not with that of Autrum, Autrum & Hoffman (1961) who found an increase in the ultra-violet sensitivity when the intensity was decreased.

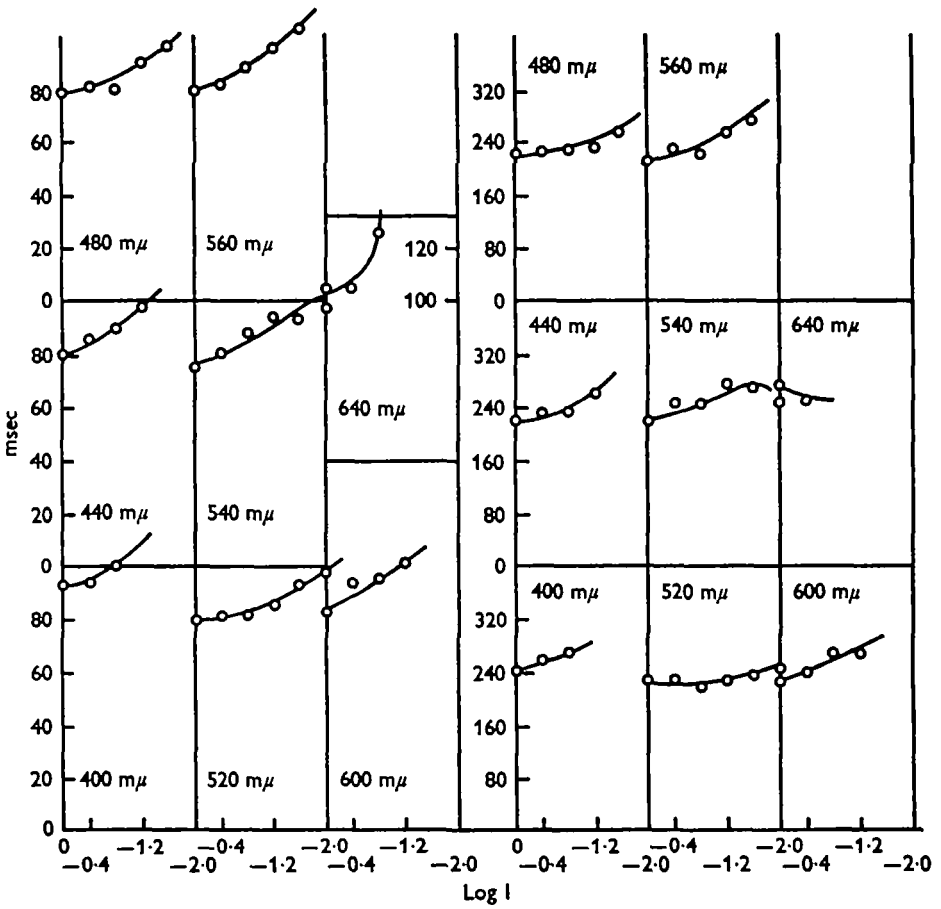

Fig. 7. Changes in the times of appearance (peak latencies) of the negative potential (four specimens, left) and the positive potential (three specimens, right) as function of light intensity and wavelength.

The spectral efficiency and sensitivity are the same in normal and in mutant specimens of both sexes (Figs. 2, 3).

The efficiency of the positive potential was found to be the same as that of the preceding negative potential (Fig. 5). Because of the variations in amplitude, response/energy curves (for the preparation of sensitivity curves) were not constructed. The relationship between response and energy was therefore calculated as percentage of the maximal amplitude (Fig. 6). The minimum intensity which gave the maximal response was at 546 mμ.

The latency of the negative and positive components of the ERG increases as the intensity decreases, and its absolute height is dependent on the wavelength (Fig. 7). Tested against wavelength, latency shows a minimum between 520 and 550 mμ (Fig. 8), and these latency curves show that the sensitivity is higher in the ultraviolet than in the long wavelengths—in agreement with the data presented for amplitude. The latency response is similar to the amplitude response, except that it is in the opposite sense. It has already been determined that the data for spectral efficiency can be obtained from these values (Yinon, 1969). Thus, a certain relationship exists between latency and amplitude that is presumably a function of the chemical and physical characteristics of the visual pigment concerned.

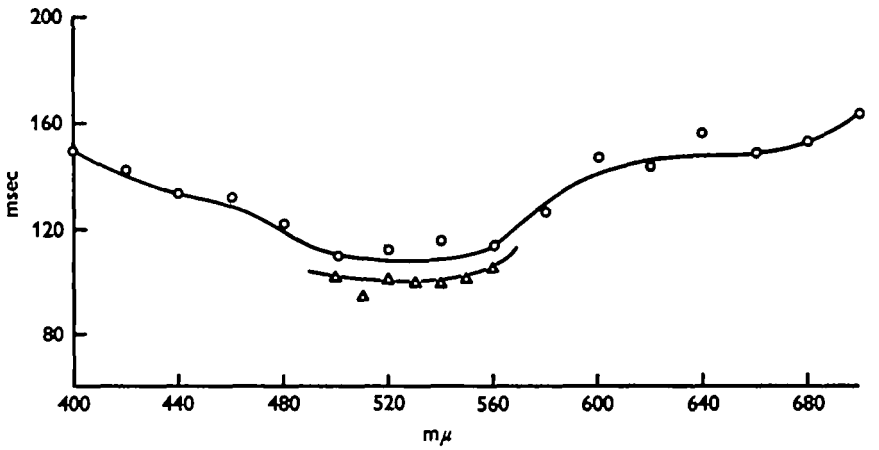

Fig. 8. Times of appearance of the negative potential (peak latencies) at various wavelengths in intervals of 20 mμ ○——○ (five animals) and of 10 mμ (in the peak region) △——△ (eight animals).

DISCUSSION

In *T. molitor* as well as in some species of field insect tested under similar conditions a peak sensitivity was obtained in the visible spectrum and also a relatively high sensitivity in the ultraviolet (Burkhardt, 1964; Wolken, 1966). The spectral response of four species of insects living in the same habitat as *T. molitor* showed peaks at 500 mμ and between 334 and 365 mμ (Stermer, 1959). Presumably, therefore, the same visual pigment is involved in this group of insects of which *T. molitor* is a representative. The question whether they have one or more visual pigments and whether colour vision is concerned demands additional research, especially in the biochemical aspect. Such investigation should be carried out also with an insect which is related evolutionarily to *T. molitor* but which is not an inhabitant of stored products.

Under normal conditions the visual pigment is not influenced by the screening pigment (Burkhardt, 1962). For instance, no difference was found between normal wild-type eye and mutant chalky eye of *Calliphora erythrocephala*. But differences in the spectral response between various eye mutants of *Drosophila melanogaster* were found by Fingerman (1952) and Fingerman & Brown (1952). These results are not contradictory because the first author examined responses in conditions of dark adaptation, in which the screening pigment was concentrated distally around the

cones and did not influence the spectral response. But absorption of light was considerable in conditions of light adaptation owing to the concentration of the screening pigment around the ommatidia in Fingerman's studies. The present experiments show that the screening pigment has no influence on the sensitivity of the compound eye in conditions of dark adaptation.

The chemical evidence for the existence of screening pigments in *T. molitor* is slight. Harmsen (1966) found only faint traces of isoxanthopterin and biopterin; Kay (1969) found that methanol extract of adult eyes fluoresces in ultraviolet with emission spectra in 323 and 378 mμ, which may point to the existence of additional screening pigments. Thus, a chemical analysis is needed to clarify what are the screening pigments.

The existence of functional screening and visual pigments in the compound eyes of *T. molitor* raises the question of what is the significance of colour vision in this insect, especially since in this species light elicits a photonegative response.

SUMMARY

1. The spectral responses of a 'stored product' insect, *Tenebrio molitor*, have been studied electrophysiologically.

2. Although primarily inhabiting a darkened environment the insect possesses a visual mechanism which could enable it to discriminate various wavelengths. The visible range for *T. molitor* lies between < 350 and 700 mμ; and the efficiency and sensitivity curves both have maxima between 520 and 550 mμ. Latency and amplitude also indicate maximum response in this same region of the spectrum.

3. Under normal conditions of dark adaptation the screening pigment does not affect the spectral sensitivity.

4. Miniature threshold potentials concealed in the normal background noise were detected by the use of the computer of average transients. The two principal components of the ERG, as well as minor components which were occasionally detected, were similarly related to wavelength. This seems to imply that the various ERG components originate in receptors of one single type.

I am indebted to Professor E. Auerbach, Vision Research Laboratory, Hadassah University Hospital, Israel, in whose laboratory and under whose supervision this work was conducted, and also to Professor A. Shulov, Department of Entomology, The Hebrew University, who provided financial support.

REFERENCES

AUTRUM, H., AUTRUM, I. & HOFFMAN, C. (1961). Komponenten im Retinogramm von Calliphora and ihre Abhängigkeit von der Spektralforbe. *Biol. Zbl.* **80**, 513–47.

BALFOUR, C. E. & CARMICHAEL, L. (1928). The light reactions of the meal worm (*Tenebrio molitor* Linn.). *Am. J. Psychol.* **40**, 576–84.

BURKHARDT, D. (1962). Spectral sensitivity and other response characteristics of single visual cells in the arthropod eye. *Symp. Soc. exp. Biol.* **16**, 86–109.

BURKHARDT, D. (1964). Colour discrimination in insects. In *Advances in Insect Physiology*, vol. 2, pp. 131–73. Eds. J. W. L. Beament, J. E. Treherne and V. B. Wigglesworth. London and New York: Academic Press.

FERWERDA, F. B. (1928). Genetische Studien am Mehlkäfer *Tenebrio molitor* L. *Genetica* **11**, 1–110.

FINGERMAN, M. (1952). The role of the eye pigments of *Drosphila melanogaster* in photic orientation. *J. exp. Zool.* **120**, 131–64.

FINGERMAN, M. & BROWN, F. A. (1952). A 'Purkinje Shift' in insect vision. *Science, N.Y.* **116**, 171–2.
HARMSEN, R. (1966). The excretory role of pteridines in insects. *J. exp. Biol.* **45**, 1–13.
KAY, R. E. (1969). Fluorescent materials in insect eyes and their possible relationship to ultra-violet sensitivity. *J. Insect. Physiol.* **15**, 2021–38.
PERTTUNEN, V. & LAHERMAA, M. (1963). The light reactions of the larvae and adults of *Tenebrio molitor* L. (Col., Tenebrionidae) and their interference with the humidity reactions. *Ann. ent. Fenn.* **29**, 83–106.
RAHMAN, K. A. & SOHI, G. S. (1939). Observations on the reactions of the dermestid beetle *Trogoderma Khapra* Arr. to light. *Indian J. Ent.* **1**, 57–63.
SNEDECOR, G. W. (1964). *Statistical Methods.* Iowa: The Iowa State University Press.
SODERSTROM, E. L. (1968). Photoactic response of adults of confused flour beetle and red flour beetle and larvae of black carpet beetle to flashes of blue-white light. *J. econ. Ent.* **61**, 973–5.
SOHI, G. S. (1966). Response of the larvae of *Trogoderma granarium* Everts (Dermestidae: Coleoptera) to different types of light. *J. Res. Punjab agric. Univ.* **3**, 166–73.
STERMER, R. A. (1959). Spectral response of certain stored-product insects to electromagnetic radiation. *J. econ. Ent.* **52**, 888–92.
TUXEN, S. L. (1956). *Taxonomists Glossary of Genitalia in Insects.* Copenhagen: Engnar Munksgaard.
WALTHER, J. B. & DODT, E. (1959). Die Spektralsensitivität von Insekten-Komplexaugen im Ultraviolett bis 290 mμ. *Z. Naturf.* **14**, 273–8.
WOLKEN. (1966). *Vision.* Illinois: C. C. Thomas.
YINON, U. (1969). Spectral efficiency as function of latency in the visual mechanism of insect (*Tenebrio molitor* L.).*Experientia* **25**, 711.
YINON, U. (1970). Similarity of the electroretinogram in insects. *J. Insect Physiol.* **16**, 221–5.
YINON, U. & AUERBACH, E. (1969). The visual mechanisms of *Tenebrio molitor*: Variations taking place in the ERG of pupa and adult during development. *J. exp. Biol.* **57**, 635–41.
YINON, U. & SHULOV, A. (1966). Some factors influencing phototactic responses of adult *Trogoderma granarium* Everts. *J. Stored Prod. Res.* **2**, 57–67.