PHOTOTACTIC RESPONSES OF SEVERAL SPECIES OF TRIATOMID BUG, VECTORS OF CHAGAS' DISEASE

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ABSTRACT

The phototactic responses of several species of triatomid bug have been investigated in the context of published information on the use of light traps as a means of collecting insects. A review of the literature indicates a considerable lack of suitable trapping methods for triatomids and so it is significant that these nocturnal insects are attracted to light. More specifically, all instars of the triatomid species studied here have manifested a distinct attraction to a comparatively new light source called betalight. Betalights consist essentially of sealed glass vessels filled with tritium. The contained tritium emits beta-particles which strike an internal coating of phosphor, this in turn producing a continuous subdued light. Betalight is a completely self-powered light source and may provide a useful attractant for triatomids in the field.

The laboratory investigation presented here was therefore concerned with elucidating those factors, both physiological and environmental, likely to influence the relative attractiveness of a betalight source.

Photoperiodic entrainment has significance in determining the timing of the triatomids' general locomotor activity and concomitantly, their diel pattern of phototactic behaviour.

The spectral composition and brightness of the betalight may be important in so far as betalights in the higher microlambert range, emitting shorter

wavelengths of radiation, elicit significantly greater responses.

The bug's levels of metabolic reserves also appear to govern the pattern of phototactic behaviour. In this connection, an investigation of the physiological significance of ecdysis, post-emergence biology and nutritional history yielded particularly valuable findings.

Of the environmental factors, ambient temperature has particular relevance. Considerably enhanced responses to betalight are elicited at more elevated temperatures. It is suggested that the level of response may be correlated with the bug's locomotor velocity, the latter bearing a linear relationship to environmental temperature.

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INTRODUCTION

1. General

The Triatominae are responsible for transmitting Chagas's disease in South and Central America. The disease organism concerned is Trypanosoma cruzi and in the advanced stage of the disease heart failure can be a major symptom as a result of the trypanosomes settling in the muscles of the heart. The disease has serious consequences for vast areas of South and Central America since a satisfactory curative therapy is not at present available. The widespread nature of the disease can be appreciated from W.H.O. figures for 1960 which estimate at least 35 million people then at risk and a total of some 7 million already infected (W.H.O., 1960).

The domestic species of Triatominae which live in close association with man - bugs thrive in substandard housing conditions - are those which are particularly important in transmission of the disease to man. The bugs feed exlusively on blood and only emerge at night from sheltered harbourages in the walls of substandard dwellings to take a blood meal. The trypanosomes undergo a developmental cycle in the bug. No trypanosomes are found in the salivary glands and indeed transmission is not brought about by the bite itself but by contamination of the wound with infective faeces. This results when the insect is crushed, or when the wound is scratched after deposition of faecal matter, or when faecal matter comes into contact with an abrasion or with mucous membranes. Perhaps the most important species involved in transmission

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The estimation of Triatomid bug populations in domestic situations presents the entomologist with a serious problem because a high proportion of the bugs will remain completely hidden until hunger causes their emergence for a blood meal. The sampling methods at present available have been outlined in a later section of this thesis and as suggested by Bertram (1971) these are not particularly reliable and where possible less time-consuming methods should be devised.

It is therefore significant that light can and will attract these nocturnal insects and in particular that a comparatively new light source called betalight, has proved successful for this purpose in laboratory investigations (Bertram 1971a; Andrewartha, 1972). Betalight has also been used elsewhere as an attractant for collecting mosquito larvae (Bertram et al., 1970). The subdued luminance of betalight which can only readily be discerned in darkened situations is produced by the action of tritium gas on a coating of phosphor which lines the inside of the small sealed glass vessel that constitutes the body of the betalight.

Betalights are advantageous in many respects; they do not require an external power supply, they glow continuously for years, they do not give out heat, and they are completely safe and unaffected by immersion in water. These advantages have proved useful in studies involving collection of mosquito larvae (Bertram et al., 1970) and more recently betalight was found to be a useful attractant for marine Crustacea (personal communication, unpublished 1973).

The present investigation is therefore concerned with the phototactic responses of various species of Triatomid bug and in particular their response to this new light source. Betalight presents the entomologist with a number of advantages which could ultimately mean its successful incorporation in a light trap of suitable design for field work. However a number of environmental and physiological factors can affect the operation of a light trap in field situations and so, using betalight as a laboratory model, the investigation has as its main aim a study of these potential factors.

2. Phototaxis

A taxis may be described as the locomotory movement of a cell or organism in response to a directional stimulus, the direction of movement being orientated in relation to the stimulus. A taxis in which the stimulus is light is therefore described as a phototaxis. Phototaxis can be positive or negative, i.e. the insect may either move towards or away from the light source. This investigation is concerned primarily with positive phototaxis, and in particular the positive phototactic responses of triatomid bugs to betalight.

The investigation also limits itself to an essentially simple phototactic response, i.e. attraction to a light source. However there are also instances where a taxis is much more complex and may be of some importance in the directional orientation of the insect. In the dorsal light reaction, for example, the tendency of an insect to orientate the head so that the dorsal ommatidia of the two compound eyes are equally strongly illuminated plays an important role in flight orientation (Goodman, 1965). Classical examples of the dorsal light reaction are shown in the Crustacea *Apus* and *Leander* (Fraenkel and Gunn, 1961) and it is also seen in certain aquatic insects such as ephemerid nymphs and dytiscid larvae which maintain their position in water by orientation to the illumination from above (Wigglesworth, 1965).

The majority of insect species manifesting phototactic responses belong to the orders Ephemeroptera, Neuroptera, Orthoptera, Coleoptera, Trichoptera, Lepidoptera, Hymenoptera, Diptera, and Hemiptera (triatomid bugs).

Phototaxis is not therefore evident in all insect groups. In general, diurnal species are not attracted to artificial light and the great majority of those species which show a marked positive response to light are indeed nocturnal.

3. Possible mechanisms underlying the attraction of an insect to a light source

The extent to which a light trap catches insects that actually detect it will depend on a number of factors. The first important factor is the amount of contrast there is between the light source and its immediate surroundings. The greater the contrast the greater the trapping effect. It has been suggested that it is indeed the factor of contrast which leads to the disturbance of the animal's normal photic orientation, resulting in the enforced 'drift' towards the light. Any phenomenon which interferes with this effect of contrast, for example moonlight or general background illumination from homes or street lamps, will considerably reduce the size of the catch (Service, 1970). The second important factor is that of intensity of the light source. Most animals will be repelled and therefore have a tendency to withdraw from the high light intensity immediately adjacent to the lamp (Service, 1970). Whereas a strong light will actually attract insects from a distance, it will almost certainly repel them as soon as they approach the light source. Indeed this kind of observation has led to considerable structural modifications or adaptations in light trap design. Breyev (1963), for example, states that U.V. light traps not equipped with suction fans resulted in very small collections owing to peculiarities in the behaviour of mosquitoes approaching a light source. The third factor which will determine the possibility of a light trap catching an insect is the extent to which the insect may be able to change from approach to avoidance, and this will ultimately depend on its flight speed. It is interesting to note that the faster heavier flyers are able to stop and change course quickly thereby avoiding capture.

The way in which an insect orientates towards a light source formed an integral part of the thought which led to the evolution of the famous Robinson Trap. This kind of careful theoretical approach is important for it must necessarily precede the formulation of any insect trap.

The Robinson theory of how an insect approaches a light source will now be considered in some detail for the same theoretical principles may indeed apply in the orientation of triatomid bugs to a light source.

At one time it was thought that insects were simply 'attracted' towards a light source and that having been attracted they would then fly to it in a fairly purposeful way. Robinson and Robinson (1950) have shown how erroneous this thinking was. First, an insect will be attracted to a relatively small light at some distance off, a light source totally isolated in a dark area. The insect will then fly towards the distant light but in the vicinity of the lamp, an area of high light intensity, the insect will tend to settle into an area of shadow. Robinson (1952) discusses this principle in explaining the merits of the Robinson Trap. It would appear that the shadow cast by the opaque funnel of the trap considerably increases the size of the catch. Insects are only positively phototactic when their eyes are in the dark-adapted condition therefore those insects which fail to enter the trap on the first approach to the light soon become light-adapted and so no longer sensitive. Many of these insects will settle in the area of shadow which surrounds the trap. Here their eyes become dark-adapted once more so that when they take to flight again and approach the light source a second time, the probability is that they will enter the trap. This phenomenon was therefore carefully considered in the formulation of the Robinson Trap.

How are insects actually attracted to light and what is the orientation mechanism? In this connection, Robinson and Robinson (1950) have considered for example, the behaviour of Lepidoptera in flight in the vicinity of a light trap. Their paper suggests that for insects a light source will produce two concentric field areas. The outermost extends from the source to a limit where the insect is still sensitive and therefore able to respond to it through its compound eyes. The innermost sphere is of smaller diameter and has been termed the "sphere of dazzle". In the outermost sphere the insect will follow a more or less direct flight path to the light source but it is within the "sphere of dazzle" that the insect is deflected from this path. In orientating towards the light source what matters particularly, according to the Robinson theory, is the way in which the facets of the compound eye are illuminated by the light source. As soon as the light is near enough to be seen by the insect, in other words to have any chemical effect on the eye, the insect will attempt to fly in such a way so as to keep the same facets illuminated all the time. This obviously leads to the insect flying in a direction which makes a constant angle with the light source. If the light is being perceived from a great distance the insect can fly in a straight line and still maintain a constant angle of flight with respect to [illegible]ght. If, however, the light is not too distant, the insect's direction of flight soon changes. In order to maintain the light shining on the same area of the eye the insect has to change direction, thereby changing its flight path. At this point, still in the outer sphere, the insect may be deflected so that instead of flying towards the light it actually flies on into the darkness. If, alternatively the insect passes somewhat closer to the light source, the direction of flight changes more rapidly and then the insect starts on the fateful "spiral path". It is only within the"sphere of dazzle" that the spiral flight will begin. The experiments carried out by the Robinson brothers also indicated that an insect which merely flies through the outermost zone and does not enter the "sphere of dazzle" will eventually pass out of the light's influence.

In connection with the important question of trap design Robinson (1952) realized that the spiral path which an insect follows when approaching a light source will bring it down until eventually it will seek a shadow area and become inactive. It was therefore important to incorporate in the trap some feature which would provide shadow areas in the trap casing. Once the compound eyes resume a dark-adapted state after the period of rest in the shadow area, the insect takes to flight and recommences its approach to the light. This feature of trap design has no doubt led to the comparative success of the Robinson Trap.

How far the Robinson theory can be applied to triatomid bugs it is a little difficult to say at this stage. However, it does seem to the author that the provision of shadow areas is a logical step, simply on the basis of what is known of the physiological mechanisms of the eye, and that it should be given some priority in the design of a trap incorporating any light source, be it betalight or the electric filament lamp.

In conclusion, the following conditions must be optimal if a light source is going to be used to collect night-flying insects (Verheijen, 1960);

i. a completely isolated source of light.
ii. light emitting wavelengths to which the insect or insects are most sensitive.
iii. a dark night without moonlight.
iv. a completely dark environment.

4. Light traps - General review of the literature

(a) Introduction

It may be as well at this stage to review the enormous literature concerning light traps since many of the points emphasised in this wealth of knowledge will have a direct bearing on the possibility of formulating betalight traps for Triatomid bugs in field situations. A great deal of this literature is descriptive and there is no need to include here an account of the wide range of light traps that have been devised for trapping insects. The summary of literature on light traps presented here (in Section 5 of the Introduction) has been included to give some idea not only of the range of traps used (a description of each trap can be found by consulting the appropriate reference) but also of the wide spectrum of taxonomic groups which have been trapped using light as an attractant.

The entomologist has necessarily evolved suitable trapping methods for insects not only to gain useful taxonomic data but also for various investigations relating to the general biology of the species under study (Kettle, 1962). The ingenuity displayed in devising suitable traps can be appreciated from Frost's (1952) review of light traps used for insect collection, survey and control. Frost's review encompasses some of the earlier traps used by entomologists and it is interesting to follow the gradual evolution away from the candle flame, the kerosene lantern, acetylene lamp, and the carbon-filament electric lamp, to the sophisticated ultra-violet lamps of today. The entomologist has needed to devise methods which will trap a sufficient number of animals for his investigation and since light has, from long experience, proved a useful attractant, light traps have, despite some failings, been used widely and successfully. In this review the uses of light traps, their advantages, disadvantages, and the kind of factors that affect their efficiency will be considered in some detail for it seems that this kind of background information is essential in formulating any kind of insect light trap.

(b) The application of light traps

Light traps have become a very important feature of practical entomology and it has been rewarding to see a shift in emphasis away from taxonomic collections by amateur entomologists to a more scientific application in biological investigations, particularly in the realm of medical entomology. Light traps, suitably positioned, may be used to forecast the outbreak of a serious medically important vector (Andreev et al.,1966). Beck (1958) operated light traps at regular intervals in different areas of Florida in order to learn something of the relative and seasonal abundance of various species of *Culicoides*. The population dynamics of the medically important disease vectors must necessarily be interwoven into all other data relating to any vector-borne disease so that a complete picture of its epidemiology may be obtained. Throughout the literature there are therefore countless references to light traps being used for the purpose of monitoring insect populations and determining their seasonal prevalence (Barr et al., 1960; Taylor et al., 1956; Moussa et al., 1966; Williams, 1965; Reuben, 1971; Hurlbut and Weitz, 1956; Davies and Williams, 1962; Murray, 1957). Going a step further it is of use to the epidemiologist to know exactly when the vector is biting and therefore transmitting the disease. Accordingly light traps have been operated to determine the nocturnal flight activity of various insect groups. Corbet (1961, 1964), by arranging mercury vapour lamps at different levels in the canopy of Zika Forest, Uganda, was able to catch and identify a vast number of specimens so that the nocturnal flight activity of 41 species of sylvan mosquito and 75 species of Tabanid could be readily assessed. The nocturnal activity of other insect groups has also been determined, notably the Tipulinae (Pinchin and Anderson, 1936), the Simuliidae of Scotland by Williams (1964) and the Noctuidae (Williams, 1935). Following on from the exact period of nocturnal flight activity, a knowledge of the vertical distribution of the species is useful. Bast and Rehn (1963) for example, determined the vertical distribution of mosquitoes in New Jersey by using light traps, and Williams (1968) investigating the Phlebotominae of British Honduras gained new information concerning the vertical distribution and flight activity of several important vector species.

Indeed Corbet's study (1964) yielded detailed information on the rhythmic activity of mosquitoes at several levels. He found that at canopy level both sexes showed a bimodal pattern of activity, females predominating in the first peak, and males in the second. This may seem very intricate data but it is important to remember that this may be particularly significant in any worthwhile epidemiological survey.

Other epidemiologically important information can be gained from operating light traps. It has been possible, for instance, to collect vast numbers of insect specimens with the view to determining a possible vector. Chamberlain et al. (1964) collected well over 25,000 mosquitoes using a C.D.C. miniature battery operated light trap. By processing these specimens and carrying out virus isolations it was possible to determine a probable vector in the St. Louis Encephalitis Virus Epidemic. Indeed light traps have proved an invaluable aid in various virus isolation studies (Hurlbut and Nibley, Jnr., 1964; Dow et al., 1964; Whitney, 1964). The quest for the vector is vitally important for it may ultimately be of use in the control or eradication measures which are finally adopted. Messersmith (1965) studying the Culicoides associated with poultry in West Virginia was able by operating light traps to pinpoint the potential arthropod vectors of Avian Infectious Synovitis. Once this kind of information is available it may then be more easy to direct control measures at the vector, e.g. a direct attack on the known breeding places.

It has already been mentioned that traps have been used to study population dynamics, species abundance etc., and in connection with this it is interesting to note that they can also reflect the relative success of local control measures (Pincus, 1938). Light traps can also indicate where and when mosquito control should be instituted in those areas where human comfort is aggravated by the presence of this biting nuisance (Vanderwerker, 1937; Mulhern, 1953b).

Although a great deal of information gained from light trap collections will be of use to the epidemiologist it is perhaps important not to forget their role in more general entomological surveys. Indeed there are many references in the literature to situations where light traps have been used simply to map the presence of certain insect species in a sampling study of a known area (Fox and Kohler, 1950; Thompson and Dicke, 1965; Thurman and Thurman, 1955; Nielsen, 1963; Lewis and Murphy, 1965).

The entomologist aiming for more general information may require experimental data concerning the kind of factors which might affect the behaviour of the insect under study and here too, light traps have found an application. Chapman and Kinghorn (1955) used their traps to determine the relationship between the flight of Scolytid beetles and local weather conditions. Williams and Davies (1957) too used light traps to assess the interrelationship between local meteorological conditions and the general activity of the Simuliidae. The entomologist may even want to assess the factors limiting the distribution of an insect and Ito's study (1964) of the seasonal abundance of mosquitoes in the four wards of Nagasaki City seems to suggest that light trap collections may also reflect the influence of the sanitation and topography of the study area.

Light traps therefore seem assured of a long and useful application in medical entomology. They are certainly a convenient means of collecting medically important insects and although many kinds of trap have been used to sample insect populations perhaps the entomologist's main aim now should be to make various modifications to those he has so as to improve their operative efficiency (Odetoyinbo, 1969). Nevertheless, light traps in combination with other methods permit a faster and more complete study of both the species composition and the numerical relationships of the medically important vectors (Zhogolev, 1959). Indeed the investigation of Collett et al. (1964) suggests that more reliable information is obtained if two collecting methods are used, e.g. light traps and larval surveys in the case of mosquitoes.

Finally, in connection with possible uses of light traps, it has been suggested that since they are an efficient and widely used means of collecting insects they could be used for the purposes of control.

Certainly, the use of light traps in controlling the medically important insects is perhaps a little difficult to envisage at this stage, particularly when considering the extensive work already done on control of agricultural insect pests. It would be presuming a great deal to imagine that the problems involved in controlling agricultural and medically important insects are in any way the same. Indeed it might be expected that control by light trap of the agricultural pest would have been a fairly straightforward matter since there is a considerable localization of the offending insect. However, at most, Stanley and Dominick's study (1958) of tobacco- and tomato-hornworm moths suggests that there was only a slight suppression of the damage to plants where light traps were used. Likewise, Ficht and Hienton (1941) have shown that infestation by the European Corn Borer could be greatly reduced but not eliminated. All other relevant work on controlling agricultural pests indicate that light traps are not a practical means of control (Pfrimmer et al., 1955; Stahl, 1954; Hervey and Palm, 1935).

(c) Factors affecting the efficiency of a light trap

Although light traps have gained an almost universal acceptance in terms of usefulness and efficiency they are nevertheless subject to changes in operative efficiency. Many factors will affect the efficiency of a trap and because some of these factors have been studied here in this investigation they will be considered in some detail. The factors affecting the efficiency of a trap are also being reviewed here because they will be of consequence in the formulation of a trap for Triatomid bugs adopting a betalight source.

First it may be as well to consider the general environmental factors affecting the efficiency of an insect trap and of these moonlight seems to be very important. Barr et al. (1960) found that the number of mosquitoes caught in a light trap on nights near full moon was only a small fraction of the numbers taken around new moon. Other investigators have obtained similar results (Horsfall, 1943; Pinchin and Anderson, 1936; Bradley and McNeel, 1935; Pratt, 1948; Huffaker and Back, 1943). Carpenter (1942) has shown that there is a cyclic variation in collections of mosquitoes which correlates very closely with the lunar phases. However, the position of the trap will determine the relative importance of moonlight for Bidlingmayer (1967) was able to show that the influence of the moon at its different phases was considerably reduced in the case of light traps situated in a forest. Corbet (1964) has similarly shown that the moon, when above the horizon, will reduce catches of mosquito above the canopy. Those below the canopy continued to be typical of nights with no moon. Indeed Provost (1959) revealed that non-attractant air sampling reveals no such cyclic periodicity.

The question of general background illumination is also important and evidence is available from the work of Barr et al. (1963) that background illumination interferes with the discrimination of light intensities by mosquitoes. The efficiency of a light trap will therefore vary in accordance with the intensity of sky illumination, and thus with the elevation of the moon and the extent to which it may be obscured by cloud and mist.

Of the meteorological factors likely to affect the efficiency of a trap, temperature is very important. Zhogolev (1959) has stated that collecting with light can only be successful when the insects are sufficiently active. This fact helps to explain why light traps are more effective in southern regions where high air temperatures favour the flight of bloodsucking insects at night. The important influence of temperature on the activity of various insect groups has been underlined in the work of Belton and Pucat (1967), Fox and Kohler (1950), and Pinchin and Anderson (1936). Indeed of all weather conditions temperature seems to be the most important (Williams, 1940). Williams (1940) has also emphasised the indirect effect of previous weather conditions for these will ultimately determine the size of the prevailing population. Hence the catch size will be dependent on two factors, namely activity and population, and these in turn will be influenced by temperature.

Rain is also a fairly important environmental factor. Belton and Pucat (1967) found that collections of Culicoides were small on rainy nights or on nights following rainfall. Rain during the day before the night collection is also associated with a low catch (Williams, 1940). The abundance of the mosquito population in Madras State, India, closely follows the rainfall pattern, for mosquito abundance as determined by a New Jersey light trap is lowest in the dry months from March to May (Reuben, 1971).

Wind can also play a role in influencing the size of a catch. Hollingsworth et al. (1961) tested the effects of setting up artificial windbreaks and showed that catches of all species were significantly increased by shielding the traps from the effects of the prevailing winds. Furthermore, it has been stated that a windspeed of around 10 m.p.h. is sufficient to reduce a night's catch to zero (U.S. Department of Agriculture, 1961).

Outside of the environment the whole question of trap design must be considered as an important influence on size of catch. It seems from all accounts in the literature that a trap will evolve by trial or error until it reaches a level of efficiency adequately meeting the needs of the investigator. Frost (1958c) was able for example to show the importance of baffles in trap design, for a trap equipped with baffles collected 6480 specimens and one without, 3552 specimens. Trapping insects by light inevitably leads to tedious sorting and so various modifications are made to the trap to exclude the larger specimens which can destroy the catch. Such modifications usually include cylindrical screens around the trap which effectively reduce the size of the catch but nevertheless relieve tedious sorting and damage (Hemmings, 1959;

Hollingsworth et al., 1961).

Differences in the quality of the light used in the trap will also have a profound effect on size and type of catch. For example, the bloodsucking Diptera appear to be more strongly attracted to ultra-violet light (Zhogolev, 1959; Kitaoka and Ito, 1964; Breyev, 1958, 1963); Hemiptera respond more freely to white light whereas Trichoptera and Microlepidoptera seem to be more strongly attracted to black light (Frost, 1953a); a fluorescent black light appears highly effective and to some extent selective for *Culicoides* (Belton and Pucat, 1967); the European Corn Borer is most attracted to the violet-blue band of the spectrum (Ficht and Hienton, 1941). Workers also report on species differences in response to different kinds of light; for example, *Aedes aegypti* responds to infra-red and is not attracted by ultra-violet or white light (Mangum and Callahan, 1968), whereas *Culex* and *Culiseta* are more attracted to a white light source and not at all to ultra-violet (Downey, 1962). The validity of such statements is perhaps open to some argument but nevertheless in certain situations light of a certain quality is far more successful for collecting a certain species of insect. Indeed Frost (1958a) suggests that since it is known that some groups or species respond more freely to ultra-violet light and others to white light, a combination of the two seems logical in order to attract a much greater variety of insects.

The importance of intensity of the light source has also been widely discussed. The number of insects caught in a light trap appears to be directly proportional to the light intensity (Barr et al., 1960; Barr et al., 1963; Belton and Pucat, 1967; Deay and Taylor, 1963; Loomis 1959; Ficht and Hientcn, 1941; Pratt, 1944). Curves representing reaction of insects to sources of radiant energy are not straight lines. At the lower end of the curve the intensity of the energy is so small that insects will not react, at the upper end the energy is so great that the insect is actually repelled (Headlee, 1937). Breyev (1963) asks why the catch increases when the intensity is increased and he concludes that it is simply an increase in the operational radius and that in those situations where the population level is low the use of a bright light source is recommended, whereas if the level is high a darker light source might be better.

The exact location of the trap is also important (Williams, 1951; Barr et al., 1960, 1963; Downey, 1962). Quite obviously a well designed trap will not function efficiently unless it is in an optimum position. Belton and Pucat (1967) consider this the most important single factor affecting the efficiency of a light trap. Ficht and Hienton (1939, 1941) have shown the importance of the position effect on light traps used to collect Corn Borer moths. They found that traps situated directly over the corn attracted a larger number of moths. Since vectors are attracted primarily by their hosts and since light traps have only a short range of influence, the effective range of the C.D.C. light trap for example being 5 m., Odetoyinbo (1969) suggests that the optimum location for sampling house-visiting mosquitoes is as near to the hosts as possible. Frost (1958b) determined the influence of height on catches by light traps and showed that the majority of insects, and generally the smaller insects, are taken in traps at the lower levels. This results from the fact that the aerial density of most insects decreases with height. Service (1970) believes that only those traps placed in the relatively narrow flight paths followed by <u>Anopheles melas</u>, for example, flying from breeding place to human habitation, will catch a large number of specimens.

So far, only extraneous factors such as meteorological conditions have been considered together with a discussion of the influence of trap design. However the situation is even more complicated because apart from these factors, the susceptibility of an animal to being caught will vary according to its behavioural and physiological attributes. This point will be considered more fully in the Results and later discussion but it would be useful at this stage to mention some of the work carried out by other investigators. For example, the nutritional state of the insect appears to be important; over 95% of the <u>Anopheles gambiae</u> caught in a light trap in Africa were unfed females (Odetoyinbo, 1969). This figure agrees remarkably well with that of Hurlbut and Weitz (1956) who state that only 5% of their mosquito catch contained blood.

Service (1969) found that the same was true for the Ceratopogonidae. Service (1970) also found that together with a large proportion of unfed mosquitoes in the catch there was also a fair proportion of gravid females. Geier (1960) has underlined the important nature of physiology in the case of the Codling Moth. The kind of trap used to catch a certain physiological age group of moth appears to be important for bait trap catches consist predominantly of half-spent females whereas light traps will collect the younger females from the population. The importance of nutritional state has already been emphasised but collections of mosquitoes by Standfast (1965) have indicated that host-seeking activity will also depress the phototactic response.

There is also a significant variation in the responsiveness of different sexes and species to trap stimuli. In many groups significantly larger numbers of one sex are likely to be caught in light traps. For example in the case of *Culicoides*, females make up the majority of light trap catches whereas males and gravid females are scarce (Belton and Pucat, 1967; Service, 1969). The same is true for Corn-Borer Moths (Ficht and Hienton, 1939). However, in the case of the crane-flies, tabanids and mosquitoes, males usually comprise a larger proportion of the catch (Pinchin and Anderson, 1936; Corbet, 1964; Ito, 1964). Corbet and Haddow (1961) found an interesting difference here for the preponderance of one sex over another was shown to depend very much on the local environment. For example, male Culicidae predominated in the catches of species found typically in the forest but females would outnumber males in catches of those species known also to bite over open ground at night. It is also interesting to note that different species respond very differently; some species of mosquito are more attracted to ultra-violet light for example (Breyev, 1963). This might be explained in terms of the spectral sensitivity of the eye or alternatively it may reflect some special optical properties of the cornea. Breyev (1963) therefore suggests the use of mercury quartz lamps whose radiant energy is greatest in the ultra-violet and green ranges of the spectrum, corresponding to the two known sensitivity peaks of the insect eye. In this way a much more varied collection of species is possible; indeed the mercury quartz lamps are said to give a more qualitative and quantitative indication of the mosquito population. Service (1970) has also underlined the species difference,for some groups are more responsive to the infra-red region of the spectrum.

The activity of an insect will be governed very precisely by its diurnal cycle and the expression of this activity will be conditioned by the prevailing climatic conditions. Therefore a light trap will only effectively catch a certain species of insect at a very specific time of day. Hutchins (1940) found one or two peaks of abundance of insect activity during the night, for example between 0100 and 0300 hours the peak of activity is reached by both the Diptera and Lepidoptera, while this period marks the nadir for the Homoptera. Murray (1957) studying the *Culicoides* of Mount Solon, Virginia, U.S.A., determined that activity was greatest during the evening and early morning hours. Clearly the diurnal cycle and the whole question of photoperiodic entrainment, which will be discussed later, are of importance in determining the period of greatest catch.

To summarise, the number of insects taken in a light trap will be influenced by three factors:

(i) *Activity of the insect*

This has been discussed in some detail. For example, light trap collections of *Aedes vexans* at dusk was not feasible because of the high level of feeding activity at that time. In light traps operated from dusk to dawn, most specimens were caught after 10 p.m. (Thompson and Dicke, 1965). Indeed Corbet (Standfast, 1965) has expressed the opinion that a large proportion of the mosquitoes caught in light traps are engaged in non-specific locomotor activity.

It is also important to consider the effect of various environmental factors on the general activity of the insect for light traps will favour not only those species which are phototactic but also those which are active during the comparatively low temperatures of night.

(ii) *Density of insect population*

The size of the catch is dependent on the actual density of population. Breyev (1963) found that the periods of larger collections of blackflies corresponded to the emergence of the new generation. Indeed Beck (1958) stresses that a light trap collection is largely accidental, depending on whether or not it coincides with an emergence.

(iii) Phototactic Response

This will depend on the design of the trap and on the behavioural and physiological state of the insect. Fox and Kohler (1950) not only underline the importance of the phototactic response but also consider the important question of whether the intensity of this reaction is constant throughout the adult life of the species. In connection with the influence of the phototactic response on the size of the catch the effective range of the light trap should also be considered. A light trap samples only a comparatively small area and therefore it can only represent the population of the immediate area and not necessarily of the geographical area as a whole (Beck, 1958). In fact there have been efforts to determine the effective range of light traps and Graham et al. (1961) for example found that Pink Bollworm Moths would respond to an ultra-violet light source up to a distance of 140 feet.

(d) Light traps incorporating other attractants

It can easily be appreciated that a great deal does in fact work against the successful operation of a light trap, if considering all the factors mentioned above. Modifications are therefore made to traps to enhance their operative success and not least of these are the addition of other attractants. Light has proved to be a successful attractant but it would seem reasonable to increase the likelihood of attracting a much larger and greater variety of insects by addition of another equally successful attractant. This point has been considered in some detail in this investigation. Carestia and Savage (1967) found that C.D.C. miniature light traps equipped with carbon dioxide cylinders attracted larger numbers and more species of adult mosquito. Newhouse et al. (1966) used dry ice in their C.D.C. light trap and increased the catch by at least four-fold, at the same time increasing the number of species by 20-25%. Reeves and Hammon (1942) also increased their catch and it seems that a combination of light and chemical attractant such as carbon dioxide is an invaluable means of obtaining more adequate samples from an area. It can be particularly invaluable for more efficient collections of vector species during certain arbovirus epidemics when large samples must be obtained quickly. However, it is important to note that male mosquitoes showing a reaction opposite to that of females are definitely repelled by the presence of carbon dioxide so that this factor should be considered seriously in light trap collections (Huffaker and Back, 1943).

The possibilities of chemical attractants in combination with light can perhaps be further broadened by the use of specific scents produced by the glands of certain insects which can effectively increase the catch (Frost, 1958a).

(e) Disadvantages encountered in light trap operations

Throughout the literature there have been controversies over the relative efficiency of light traps. Some workers have found that in certain situations light traps did not prove a satisfactory method of sampling the local insect population. For example, Reuben (1971), Roberts (1965), and Hu and Grayston (1962) have resorted to steer-baited traps in preference to a light trap. Light traps may be unpopular for several reasons and a few of these have been listed below: the principal disadvantage appears to be the large catch of other insect orders that is invariably taken and the resulting tedious sorting (Service and Boorman, 1965; Pritchard and Pratt, 1944); second, animal host attraction may be more constant than that of light since light traps have a cyclic effectiveness determined by moonlight (Harwood, 1961); third, animal-baited traps do not require a power source so that they will be widely used in preference to the light trap in those situations which lack extensive power supplies (Pritchard and Pratt, 1944); finally, a light trap is obviously useless for the day-flying species of insect which may be equally important in the transmission of a disease (Kettle, 1962). These are a few of the disadvantages of light traps which are often mentioned, but in the end the advantages seem to outweight the disadvantages (Mulhern, 1953b) and these will now be considered in some detail.

(f) Advantages presented by light traps

Beck (1958) in his collection of Culicoides found that a light trap was comparatively inexpensive to operate, that it required little of the collector's time, that the efficiency of the trap is the same in different areas giving a 'standard' collection at different times and in different localities, and that although a light trap is definitely selective with respect to phototaxis, it is nevertheless random in respect of commonness, i.e. it does not matter whether the insect is common or rare. Pritchard and Pratt (1944) in their collection of mosquitoes listed further advantages; an animal-bait trap will only collect females in search of a blood meal whereas a light trap will collect males and females; second, attraction to a light trap affords some measure of standardization through the use of bulbs of given wattage; third, a light trap can be operated with an automatic time switch which means nightly collections throughout the season at low cost.

There are various instances cited in the literature where a light trap was operated more successfully than artificial shelter traps, animal-baited traps or suction traps (Hurlbut and Nibley, 1964; Service, 1969; Williams, 1965). Certainly light traps would seem much better than those traps depending on human skill, since the latter have been found subject to considerable variation (Vanderwerker, 1935). Indeed Mulhern (1953a) suggests the possibility of obtaining better results with light traps by standardizing their mechanical performance.

In comparing the results obtained using light traps with those involving other methods some very interesting differences are notable. Moussa et al. (1966), for example, discovered that although light traps collected more adult mosquitoes than did resting boxes, the latter showing only one of five peaks of abundance, resting boxes collected a larger number of engorged females. There also appears to be a seasonal factor in the relative efficiency of each trapping method, for larger numbers of different mosquito species were taken in light than in chick-baited traps during the summer months from April to September whereas the reverse was true in the cooler months from October to March (Taylor, Meadows and Baughman, 1966).

The views about the relative efficiency of light traps appears to be widely divergent, some workers considering that they yield a more uniform index than do most other collecting methods and that they are probably the best method for sampling an insect population in any given area (Sun, 1964; Love and Smith, 1957; Beck, 1958), whereas some workers do not appear to favour their extensive operation. Perhaps Provost (1959) views the application of light traps more realistically for he considers that the value of any light trap collection is proportional to what is known of the trap's abilities, limitations, and eccentricities

5. Summary of literature on light traps, with particular reference to insect orders caught

TAXONOMIC GROUP CAUGHT	NAME OF TRAP (with specifications)	AUTHOR(S)
LEPIDOPTERA	Ultra Violet Light Traps	Ficht & Heinton (1939) Frost (1958a) Graham, Glick & Hollingsworth (1961)
	Transparent Light Trap	Common (1959)
	Mercury Vapour Light Trap	Geier (1960)
	Minnesota Light Trap (White light)	Frost (1958a)
	Minnesota Light Trap (Black light)	Frost (1953a)
	Pennsylvania Insect Light Trap (equipped with black light fluorescent lamps)	Frost (1957, 1958b)
	Robinson Trap	Robinson & Robinson (1950)
	Rothamsted Trap	Williams (1948, (1951)
COLEOPTERA	Ultra Violet Light Traps	Frost (1952. 1958a)
	Window Flight Trap	Chapman & Kinghorn (1955)
	Pennsylvania Insect Light Trap (equipped with black light fluorescent lamps)	Frost (1958b)
	Hungerford Subaquatic Light Trap	Hungerford, Spangler & Walker (1955)
	Robinson Trap	Southwood(1966)

TAXONOMIC GROUP CAUGHT	NAME OF TRAP (with specifications)	AUTHOR(S)
TRICHOPTERA	Ultra Violet Light Traps	Frost (1958a)
	Minnesota Trap (Black light)	Frost (1953a)
	Pennsylvania Insect Light Trap (equipped with black light fluorescent lamps)	Frost (1957)
	Hungerford Subaquatic Light Trap	Hungerford, Spangler & Walker (1955)
ODONATA	Hungerford Subaquatic Light Trap	Hungerford, Spangler & Walker (1955)
HYMENOPTERA	Pennsylvania Insect Light Trap (equipped with black light fluorescent lamps)	Frost (1958c)
HEMIPTERA	New Jersey Light Trap	Frost (1952)
	Ultra Violet Light Traps	Frost (1958a)
	Minnesota Trap (White light)	Frost (1953a)
	Pennsylvania Insect Light Trap (equipped with black light fluorescent lamps)	Frost (1958b)
	Hungerford Subaquatic Light Trap	Hungerford, Spangler & Walker (1955)
	Rothamsted Trap	Williams(1940)

TAXONOMIC GROUP CAUGHT	NAME OF TRAP (with specifications)	AUTHOR(S)
DIPTERA Suborder NEMATOCERA	Sodium Vapour Lamps	Service & Boorman (1965)
Family Culicidae (Adults)	New Jersey Light Trap	Hurlbut & Nibley (1964) Hurlbut & Weitz (1956) Love & Smith (1957) Pippin (1965) Reuben (1971) Sun (1964) Taylor, Work, Hurlbut & Farag Rizk (1956) Whitney (1964) Bast & Rehn (1963) Horsfall (1943) Thurman & Thurman (1955) Huffaker & Back (1943) Downey (1962)
	Ultraviolet Light Traps	Breyev (1958, 1963) Downey (1962) Kitaoka & Ito (1964)
	C.D.C. Miniature Light Trap	Carestia & Savage (1967) Odetoyinbo (1969) Chamberlain, Sudia, Coleman & Beadle (1964) Taylor, Meadows & Baughman (1966) Bertram (1971b)
	C.D.C. Miniature Light Trap (infra-red radiation)	Mangum & Callahan (1968)
	Mercury Vapour Light Trap	Corbet (1964) Corbet & Haddow (1961)
	Minnesota Trap (White Light)	Loomis (1959)
	Monk's Wood Trap	Service (1970)

TAXONOMIC GROUP CAUGHT	NAME OF TRAP (with specifications)	AUTHOR(S)
DIPTERA Suborder NEMATOCERA		
Family Culicidae (Adults)	American Model Light Trap (equipped with 25 watt white inside frosted bulb	Loomis & Hanks (1959)
	Miniature Battery Operated Light Trap (Sudia & Chamberlain 1962)	Dow, Coleman, Meadows & Work (1964)
	Paraffin Lamps	Service & Boorman (1965)
Family Culicidae (Larvae)	Betalight Trap	Bertram, Varma, Page & Heathcote (1970)
	Hungerford Subaquatic Light Trap	Hungerford, Spangler & Walker (1955)
Family Tipulidae	New Jersey Light Trap	Frost (1952)
	Rothamsted Trap	Pinchin & Anderson (1936)
	Pennsylvania Insect Trap (equipped with black light fluorescent lamps)	Frost (1958b)
Family Psychodidae	Miniature Battery Operated Light Trap (Sudia & Chamberlain 1962)	Williams (1968)
Family Chironomidae (Adults)	New Jersey Light Trap	Frost (1952)
(Larvae)	Hungerford Subaquatic Light Trap	Hungerford, Spangler & Walker (1955)
Family Ceratopogonidae	New Jersey Light Trap	Kohler & Fox(1951) Murray (1957) Service (1969)
	New Jersey Light Trap (equipped with fluorescent black light)	Belton & Pucat (1967) Rowley & Jorgensen (1967)

TAXONOMIC GROUP CAUGHT	NAME OF TRAP (with specifications)	AUTHOR(S)
DIPTERA Suborder NEMATOCERA		
Family Ceratopogonidae	Ultraviolet Light Traps	Kitaoka & Ito(1964) Zhogolev (1959)
	Rothamsted Light Trap	Service (1969)
	Light Trap (unspecified)	Messersmith (1965) Nielsen (1963)
Family Simuliidae	New Jersey Light Trap	Frost (1952)
	Ultraviolet Light Trap	Breyev (1963)
	Rothamsted Light Trap	Davies & Williams (1962) Williams & Davies (1957)
Suborder BRACHYCERA		
Family Tabanidae	New Jersey Light Trap	Frost (1953b)
	Mercury Vapour Light Trap	Corbet (1964)
Suborder CYCLORRHAPHA		
Family Drosophilidae	Ultraviolet Light Trap	Frost (1958a)
Family Muscidae	Ultraviolet Light Traps	Deay & Taylor(1963)
	White Light Bulb	Buxton (1955)
EPHEMEROPTERA	Hungerford Subaquatic Light Trap	Hungerford, Spangler & Walker (1955)
SIPHONAPTERA	Flea Trap	Frost (1952)

6. Trapping of Triatomid Bugs

A great deal has been said concerning light traps for collecting insects generally, but at this stage the possibility of using light traps for catching Triatomid bugs in field situations should be considered.

The limitations and eccentricities of light traps have been discussed in some detail but probably the collection of triatomid bugs presents the entomologist with even more real and difficult problems. The domestic species of triatomid, and certainly the most important epidemiologically, spend the greater part of their lives in cracks and fissures in the walls and roofs of primitive dwellings and animal habitations. From these dark sheltered places the bugs emerge only at intervals to take a blood meal and, having gorged, they return to these habitats. Where housing conditions are primitive, where mud or adobe walls and thatch roof are used, conditions are ideal for the bugs. The bugs have therefore presented the entomologist and epidemiologist with significant problems in terms of trapping and collecting methods. An important aim in the present epidemiological investigation of Chagas's disease in South America is to formulate a means of furnishing more quantitative data on relative bug densities inside houses so that the epidemiology of the disease can be more easily resolved. The epidemiologist requires sufficient and reliable quantitative data to interpret the relative importance and range of the disease. The entomologist therefore needs to devise a suitable means of trapping and collecting the bug vectors so that he can readily supply this important information.

As yet no really satisfactory method for trapping the bugs seems to have been designed. The methods available for collecting the bugs are also inadequate in many respects and very time-consuming. The bugs' sheltered habitat means they are extremely difficult to get at and the entomologist is confronted with an active search for the bug vectors during the day. One of the techniques presently employed involves blowing pyrethrum powder into the crevices of the bug habitat in the hope that the bugs are sufficiently irritated to cause them to emerge (Cancado, 1968). Pipkin (1968) in his investigation of domiciliary reduviid bugs in Panama emphasises the difficulties presented by daytime searches and concluded that pyrethrum dusts or aerosol insecticidal 'bombs' used to flush out the bugs were relatively unproductive for Rhodnius pallescens. Alternatively, perforated boxes may be attached to the walls of the dwelling which ultimately may trap any bugs that emerge voluntarily (Gomez Nunez, 1965). This is not particularly reliable as a trapping method although it is interesting to note that Pipkin (1968) finally adopted night collections when the bugs either emerged voluntarily to obtain a blood meal or were sufficiently disturbed by the light shone into their crevice to cause their emergence.

Clearly better sampling methods are warranted not only for epidemiological purposes but also in vector control programmes where an indication of bug infestation can ensure a more economic application of insecticides. Does light therefore present a useful prospect for trapping bugs? The answer to that seems to be reassuring for there are references in the literature to various species of triatomid bug being attracted to a light source of some kind or another. Correa (1968) reports that sylvatic species of triatomid, commonly living in palms and other arboreal environments will sometimes enter lighted dwellings. Wood and Wood (1965) report on the attraction of sylvatic species of Californian traitomid to roadways illuminated by mercury vapour street lamps. There is perhaps good reason to suppose that the triatomids invading the residential areas of southern California were ultimately attracted by the lighted dwellings (Wood, 1951, 1954). Special traps incorporating light as an attractant have been operated in order to catch bugs and have been fairly successful. Wood and Anderson (1965) describe a procedure used to collect <u>Triatoma</u> <u>p. protracta</u> at a boys' camp in Los Angeles. A white sheet soiled with dirt to create a dull non-reflecting surface was nailed to a wooden frame and behind this was placed a 15 W fluorescent blue light having a wavelength peak of 4400 A. The bugs which were attracted to the sheet were collected by hand. There are also reports of triatomids being attracted to black light in outdoor traps (Ryckman et al., 1965; Sjogren and Ryckman, 1966). Ventocilla and Silva (1968) have reported the finding of specimens of three species of blood-sucking assassin bugs, including

Panstrongylus megistus, in a black light trap which had been used to study the fluctuations in levels of insect population in a cocoa field of Itabuna, Bahia.

Sjogren and Ryckman (1966) extended the objectives of their light-trapping to include determination of the period of flight activity, both daily and seasonal; assessment of the influence of various environmental and physiological factors on flight activity; and formulation of a light trap technique which could control inexpensively the kissing bug population causing annoyance to residents in California.

More recently Bertram (1971a) has reported on the diel rhythm of attraction of triatomid bug vectors of Chagas's disease to betalight. (Betalights are described in detail in the Techniques section of this thesis). Betalight, as a comparatively new light source, may have considerable potential in the field situation as an attractant which could be incorporated in a trap of suitable design. The investigation presented here has as its main aim a full consideration of factors likely to affect its efficient functioning if and when operative in field studies. Alternatively, betalight may play a considerable role in studies of bug responses to light in laboratory research.

TECHNIQUES

1. Laboratory culture of Triatomid bugs

The bugs used during the course of this investigation were taken from the stock colony in the Department of Entomology of the London School of Hygiene and Tropical Medicine. The rearing techniques adopted for each species will be outlined briefly.

(i) *Rhodnius prolixus*

Adult bugs of *Rhodnius prolixus* are kept in large 1 lb jars, the tops of the jars being sealed with nylon gauze. Each jar is equipped with a piece of folded blotting paper, the latter providing a suitable surface on which the bugs can rest and, secondly, an absorbent for the aqueous excrement. Male and female bugs are kept in the same jar.

All nymphal stages are kept in flat-bottomed glass tubes (5 cm. x 3 cm.), the tubes being sealed with nylon gauze.

Adults and nymphs are given a blood meal once every two weeks from the ears of lop-eared rabbits. The rearing jars and tubes are attached to the ears of the rabbit and the contained bugs are thus enabled to obtain a blood meal simply by extension of the proboscis through the nylon gauze. Generally speaking, one blood meal is sufficient for each nymphal stage to moult through to the next, but the 5th stage nymphs may require a second blood meal to reach the adult stage.

The insectary in which the *Rhodnius prolixus* colony is kept has the following environmental conditions: a temperature of 26 ± 1°C. and a relative humidity approximating to 70%.

These rearing techniques were used by Buxton and are described by him in his paper (Buxton, 1930).

(ii) Triatoma maculata

Fourth and 5th instar nymphs and adults of Triatoma maculata are kept in Kilner jars whose tops are sealed with screw-cap metal gauze lids. The jars contain folded pieces of blotting paper which not only serve to absorb excrement but also serve as a surface on which the bugs can rest.

First, 2nd and 3rd instar nymphs are kept in flat-bottomed glass tubes (5 cm. x 3 cm.).

The bugs are fed once every two weeks and in the case of the 1st, 2nd, and 3rd instar nymphs the blood meal is obtained from rabbits' ears whereas for the 4th's, 5th's, and adult stages the blood meal is taken from the general body surface of a guinea pig.

The insectary in which these bugs are reared has a temperature of 28 ± 0.5°C. and a relative humidity ranging from 40-60%.

(iii) Triatoma infestans

Rearing techniques are the same as those mentioned for Triatoma maculata.

(iv) Triatoma phyllosoma

The method for supplying the blood meal is slightly different here. Prior to feeding the bugs are placed in special Geigy cages. A Geigy cage used in this feeding procedure consists essentially of a rectangular wire framework covered over with nylon. These cages can be applied to restrained rabbits or guinea pigs. After the bugs have taken their blood meal the cages are laid flat on sheets of blotting paper so as to absorb the copious, largely aqueous, excrement produced shortly after feeding. The bugs are then removed from the Geigy cages and transferred back to Kilner jars.

(v) Panstrongylus megistus

Rearing techniques are the same as those already described for Triatoma maculata and Triatoma infestans.

There are inevitable variations in the techniques adopted by various entomologists for maintaining colonies of Triatominae (Ryckman, 1952). However the techniques described above have been successful and indeed have provided the author with sufficiently large numbers of bugs for experimental work.

Finally, it is important to note that the insectary in which the various experimental work was carried out was completely separate from the insectaries used for rearing of the colonies.

2. Betalights.

Betalights consist essentially of small sealed glass vessels which are filled with tritium gas. The vessels are lined internally with a coating of phosphor. The contained tritium, a radioactive isotope of hydrogen, on decay emits beta-particles (high energy electrons) which strike the internal coating of phosphor. The beta-particles are absorbed by the phospher, the phospher coating then emitting a continuous subdued light. The light which is produced is in the visible region of the spectrum but is not readily detectable in daylight or artificial illumination.

The colour of the light emitted by the betalight is dependent on the phospher used in the internal coating.

Betalights (manufactured by Saunders - Roe Developments Ltd., Hayes, Middlesex) although marketed now for several years, are perhaps now finding a wider application as sources of low-level illumination. They can be readily used to illuminate emergency exit signs and have found a useful application in the illumination of aircraft panels. As a low-level light source they incorporate a number of advantages; they are a completely self-powered light source, glowing continuously, without need of an external energy source or any kind of maintenance. They are totally reliable and are said to have a useful life of over twenty years.

Initially, betalights might appear expensive but when bearing in mind the advantages just listed they are in fact a comparatively cheap light source. Tritium is unfortunately expensive and this of course accounts for the greatest part of the cost of any betalight. However the manufacturers do recommend that the individual take care in his selection ensuring that the light chosen is no bigger or brighter than that necessary for the job for which it is required. Despite the expensiveness of the tritium gas, it was selected from a large number of possible radio-isotopes because as such it incorporates the best combination of safety, cost, life and efficiency.

Betalights come in a wide range of shapes, sizes and colours. The various shapes include straight tubes, curves, discs and spheres, although they can be tailor-made for the individual as far as glassblowing techniques will allow. A wide range of colours within the visible spectrum are also available but unfortunately they cannot be produced at exactly the same levels of brightness.

The question of brightness is an interesting one, for the brightness of a betalight is determined by two factors. The apparent brightness of any betalight to the human eye results, firstly, from the actual betaflux incident on the phosphor surface (thus the total amount of tritium present in the glass vessel is important) and secondly, the wavelength. The betaflux incident will be dependent on the tritium/phosphor area ratio, and the higher this ratio is, the greater will be the brightness of the betalight. As mentioned previously the colour produced by the betalight will depend ultimately on the kind of phosphor used. Colours covering a wide range of the visible spectrum can be produced by the manufacturers, but green and yellow are widely used since these appear brightest to the human eye. The eye is most sensitive to light in the green and yellow region of the spectrum and sensitivity decreases in both the red and the blue. This phenomenon is said to account for the apparent greater brightness of the green and yellow region wavelengths. The relative brightness of certain wavelengths in the betalight range is shown in the table below where it will be seen that if a betalight of any given size has a maximum brightness of 100 microlamberts in the green wavelength then a red light of the same size can only have a brightness of 15 microlamberts.

Colour	Red	Deep Orange	Orange	Yellow	Green	Blue	White
Symbol	R	D	O	Y	G	B	W
Dominant Wavelength mμ	608	592	586	570	540	473	
Relative Brightness	15	60	65	110	100	30	75

The various brightnesses of the betalights manufactured are given in microlamberts (all measurements refer to brightness, not total light, so the units are in lamberts not lumens) but approximate conversion factors to other units are listed in the technical literature distributed by the company. It is interesting to note in connection with the question of brightness that levels of brightness up to 4000 microlamberts can be produced for individual requirements.

A suitable classification system for the wide range of betalights produced by the company is described in the technical literature. There are several parameters which define a betalight; first, its size and shape, second, its colour, and third, the brightness. The type number specification of a betalight is therefore divided into 3 sections:

shape & size / colour / brightness

As an example, a betalight widely used in the investigation had the following type number specification:

S10/G/2000

This immediately gives an indication of size and shape, in this case spherical, second, the colour which is green and third, the brightness which is 2000 microlamberts.

Although betalight is a radioactive light source there is no danger of any external radiation because the glass is impervious to tritium and absorbs any of the beta-radiation which has not already been absorbed by the lining of phosphor. At the same time betalights have been designed to withstand full military and aircraft shock, they are non-incendive, and are unaffected by immersion in oil, water or the majority of the corrosive materials. They are therefore a totally reliable light source and the fact that they have been used for the panel illumination of spacecraft in the Apollo moon shots would seem to indicate that they will find a much wider application in the years to come.

3. Experimental Apparatus

(a) Polythene choice chamber

The polythene choice chamber was specially designed by Bertram and differs only slightly from that used in his original investigation (Bertram, 1971a). It consists of an opaque white polythene box (31 cm. x 20 cm. x 10 cm. deep) which forms the central chamber. The central chamber is covered with a removable lid which is kept in place throughout the experiment. The lid is specially constructed to include a clear perspex viewing screen which facilitates easy observation of the bugs, thereby obviating the need for continual removal of the polythene lid. The viewing screen has a central aperture (5 cm. diameter) which can be closed by means of a perspex flap (see Figures 1, 2 and 3).

At each end of the central chamber, in the mid-line, are attached two semi-transparent polythene bottles (6 cm. x 6 cm. x 13 cm.). An aperture (2 cm. diameter), big enough to allow the passage of bugs, connects the central chamber to the polythene bottle in each case. The connecting aperture is at a height of 1 cm. from the floor of the chamber. A short plasticine slope leads from the floor of the chamber to the aperture. The polythene bottles are attached in such a way that the mouth of the bottle is pointed vertically downwards. The mouth of the polythene bottle is connected to a small clear perspex container (7 cm. diameter, 6 cm. deep). The perspex container is easily screwed onto the polythene bottle. The bugs are thus able to pass from the central chamber into the polythene bottle and finally drop into the perspex container. The polythene bottles together with their perspex containers act as traps at either end of the apparatus. Once

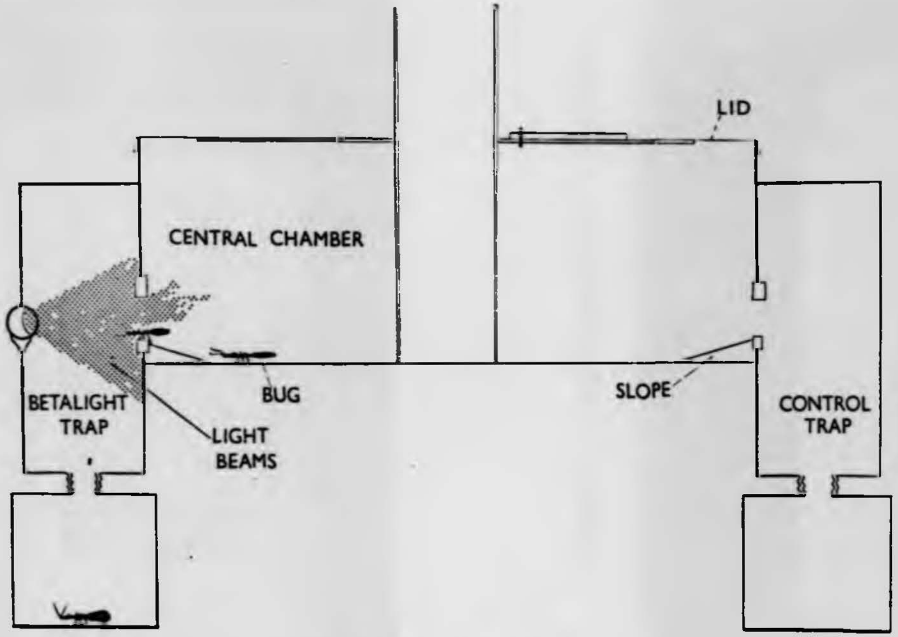


FIGURE I. CHOICE CHAMBER

Figure 2. Polythene Choice Chamber

Figure 3. Polythene Choice Chamber

the bugs have fallen into the trap, the perspex container can be unscrewed and the bugs easily removed from the trap.

A betalight of any type number specification can be attached to one of the polythene bottles, thereby forming a betalight trap. The betalight is fixed with adhesive tape to a hole in the polythene bottle and is positioned so that it is in line with the aperture connecting the central chamber and the trap. The betalight, which glows continuously, therefore shines through the aperture into the central chamber. The polythene bottle at the opposite end of the chamber is without light (unless stated otherwise in the experiment described) and constitutes a control trap.

The chamber is also equipped with a long perspex tube (5 cm. diameter, 16 cm. long) which can be inserted into the aperture of the viewing screen. The perspex tube reaches down to the floor of the central chamber. The bugs are introduced into the central chamber via the perspex tube, the contained bugs being unable to escape into the rest of the chamber. When all the bugs have been introduced the perspex tube is carefully removed from the central chamber, thereby releasing the bugs. A perspex flap attached to the viewing screen is positioned over the aperture in the screen, not only sealing off the chamber to prevent escape of bugs, but also keeping environmental conditions within the central chamber fairly constant.

An Edney hygrometer and a mercury thermometer were attached to the upper part of the inner surface of the wall of the chamber so that environmental conditions could be noted and recorded where appropriate.

(b) Glass choice chamber

The glass choice chamber consists of a long glass cylinder, 28 cm. long, with a diameter of 4 cm. The floor of the central chamber has a plaster of paris lining to a depth of 0.5 cm. The floor of the chamber has a total length of 20 cm. The plaster of paris lining provides a suitably flat surface to facilitate locomotion of the bugs. At each end of the apparatus the floor of the chamber falls vertically into a lateral glass tube which leads directly into a small glass bottle. The glass bottle and the lateral glass tube have ground glass connections thereby facilitating easy removal of the glass bottles which constitute traps at either end of the chamber.

On top of the choice chamber, half way along, is an aperture of 2 cm. diameter through which the bugs can be introduced into the apparatus. The chamber is equipped with a glass introduction cylinder and this is inserted into the aperture. The introduction cylinder reaches right down to the floor of the chamber. Bugs are introduced into the central chamber via the cylinder, the contained bugs being unable to escape into the rest of the chamber. When all the bugs have been introduced, the cylinder is carefully removed thereby releasing the bugs at a time convenient to the investigator. The chamber can then be sealed by means of a rubber bung.

A betalight was taped to one end of the apparatus, and this together with the lateral glass tube and glass fall bottle constitute a betalight trap. The other unilluminated end of the apparatus constitutes the control trap.

To determine a diel rhythm of attraction to betalight in 1st and 2nd instar nymphs, the experimental procedure which has been outlined in a later section of the thesis was followed. Essentially the procedure consisted of noting the total number of bugs which entered the betalight trap and control trap at hourly intervals. The bugs were then returned to the central chamber via the glass introduction cylinder.

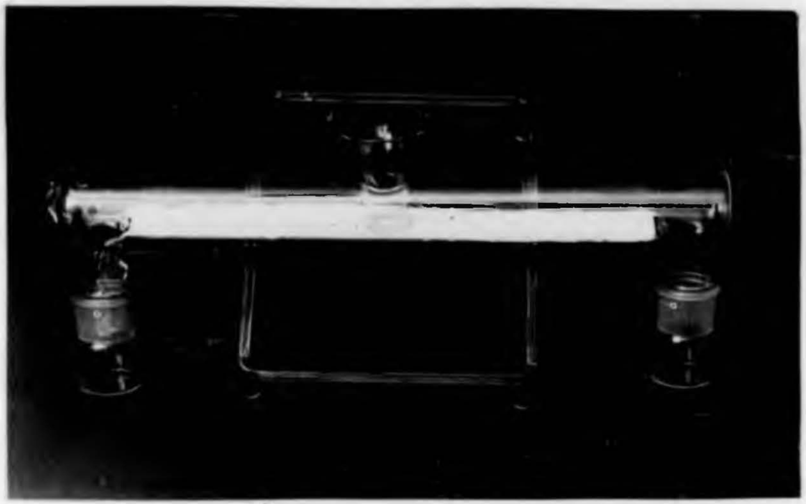


Figure 4. Glass Choice Chamber

(c) Photoperiodic Entrainment Chamber

A chamber for the photoperiodic entrainment of the bugs was a prerequisite for the investigation. Originally the author had to watch the bugs' behaviour over a 24 hour period in order to estimate the onset and duration of attraction to the betalight source during this time (Andrewartha, 1972). Since a certain cycle of light and dark can govern the onset and duration of the general activity of the bugs it seemed reasonable to "switch" the bugs' diel rhythm of activity so that it corresponded to a more convenient working schedule during the day instead of endless observations throughout the night.

Photoperiodic entrainment was shown to be feasible and the bugs showed their greatest activity during the day (remembering that in the earlier investigation the peak of activity occurred in the early hours of the morning).

The apparatus used for the purpose of photoperiodic entrainment consisted of a large light-proof wooden box (2'6" x 2'6" x 2'6"). An atlas 8W warm white strip light was placed inside the light-proof chamber, and this provided the bugs with their time-cue or zeitgeber. The light was connected to a Venner Auto Point time switch so that a carefully arranged cycle of light and dark could be set automatically. Generally, unless stated otherwise, the following photoperiod was adopted: a scotophase (or dark period) from 1300 - 1500 hours and a photophase (or light period) from 1500 - 1300 hours, in all 2 hours of light and 22 hours of darkness.

The chamber was kept in an underground insectary (at the London School of Hygiene and Tropical Medicine) in which the temperature and relative humidity were fairly constant. To maintain a fairly steady relative humidity within the chamber itself a large enamel tray was kept continuously filled to the brim with water. Continuous thermohygrograph records were kept and periodically checked against a mercury thermometer and whirling hygrometer.

Bugs were kept in Kilner jars equipped with special screw-cap metal gauze lids. A piece of blotting paper was placed inside the Kilner jar so as to absorb faecal material and at the same time to provide the bugs with a surface on which to rest. The Kilner jars, containing bugs ready for photoperiodic entrainment, were then placed inside the chamber.

Special glass racks, able to support several Kilner jars, were placed in the water-filled enamel tray. The jars, positioned over the water tray, were therefore maintained in a warm environment with an equable relative humidity. The chamber was maintained at 27 ± 4°C. and 85 ± 5% relative humidity. At the same time the strip light, automatically controlled by a time switch, provided the bugs with a time cue, or zeitgeber, in the form of definite cycles of light and dark. The bugs were therefore photoperiodically entrained and so the onset and duration of their activity could be governed very precisely to suit the needs of the investigator.

(d) Apparatus used to measure the diel pattern of activity of bugs

Daily rhythms of locomotor activity can be estimated by using a number of techniques (Cloudsley-Thompson, 1961). The simplest method is of course that of direct observation of the insects but this is necessarily time-consuming and a serious disadvantage of this method results from the need to illuminate the insects during their scotophase. However, with more sophisticated techniques at the disposal of the entomologist, automated recording of insect activity has become possible. The instrumentation involved in automated recording of insect activity needs to meet two requirements:

(1) the slightest movement of the insect needs to be detected and transduced into a recording system;

(2) the activity patterns need to be recorded on a suitable time scale.

These requirements are best met by an actograph which can be connected to a suitable recording device. In this investigation the actograph which was employed was very similar to that used by other investigators (e.g. Harker, 1956) who have been looking at the behavioural activity patterns of various other insect groups.

Essentially an actograph is no more than a light-weight cage which is delicately balanced on a knife edge. An insect is confined in the cage and any movements by the insect will cause the cage to move from side to side. The movements of the balanced cage open and close a mercury dip switch and the resulting making and breaking of contacts are recorded on either a kymograph or strip chart recorder.

In order to estimate the daily activity patterns of triatomid bugs it was decided to set up an actograph. The actograph used here consisted simply of an oval-shaped cage. The cage was constructed from a light wire framework, the entire framework being covered over with nylon net. The use of these materials ensured a simple light-weight actograph cage. The whole cage was delicately balanced on a knife edge so that any movements by a bug contained within the cage caused the actograph to tip to one side or the other. Two small pieces of wire positioned diametrically at both ends of the actograph cage were so arranged that on tipping to one side or the other the wire would brush through a mercury dip switch. In this way, an electric circuit can be broken or completed according to the movements of the actograph cage. Recordings of activity were then made automatically by a special Servoscribe Pen Recorder (manufactured by Smiths Industries) connected to the actograph.

This method proved a reliable and successful means of determining the activity patterns of bugs for at the end of the observation period it was only necessary to examine the chart produced by the pen recorder to estimate the onset and duration of maximum activity.

(e) Apparatus used to determine the speed of locomotion in Triatomid bugs

The apparatus used to determine the speed of locomotion in triatomid bugs was necessarily simple. It consisted of a long glass rectangular chamber, 5 cm. wide by 30 cm. long. The chamber had a depth of 12 cm. One end of the chamber was connected to a glass cylinder which had a diameter of 9 cm. and a depth of 12 cm. The connecting aperture between the chamber and the cylinder was 2 cm. by 3 cm. The other end of the chamber was completely closed off by a glass face with a width of 5 cm. and a height of 12 cm.

The floor of the chamber was covered with filter paper to assist locomotion of the bugs. A distance of 22 cm. was arbitrarily marked out on the floor of the chamber so that the time taken for a bug to cover this distance could be noted and thence the speed of locomotion calculated.

(f) Wavelength Discrimination Chamber

The design of the Wavelength Discrimination Chamber used in this investigation was based on that used by Kimmins (1971) in his observations on wavelength preferences of the Bark beetle. The apparatus consisted essentially of a glass triangular Choice Chamber connected at its apex to a glass cylinder. The base of the triangular Chamber had a length of 26 cm. and the sides were 37 cm. long. The Choice Chamber was connected to a glass cylinder which had a diameter of 7 cm. The glass cylinder constituted the Releasing Chamber. The Choice Chamber and the Releasing Chamber had a depth of 12 cm.

The connecting aperture between the Choice Chamber and the Releasing Chamber had a width of 3 cm. and a height of 2 cm.

Towards the basal end of the triangular chamber were 4 traps which contained the betalights. Each trap had a width of 5 cm. and a length of 8 cm. The aperture connecting the Choice Chamber and each Betalight Trap had a height of 2 cm. and a width of 1.5 cm. The traps were given numbers, 1-4, and betalights could be easily removed from one position to another (See figure 5). This enabled a position change for each betalight at hourly intervals thereby eliminating unnecessary bias on the part of any one of the traps.

The entire apparatus was painted over with black designers' acrylic gouache thereby ensuring that each Betalight Trap was completely light-proof and at the same time providing a dull black non-reflecting surface.

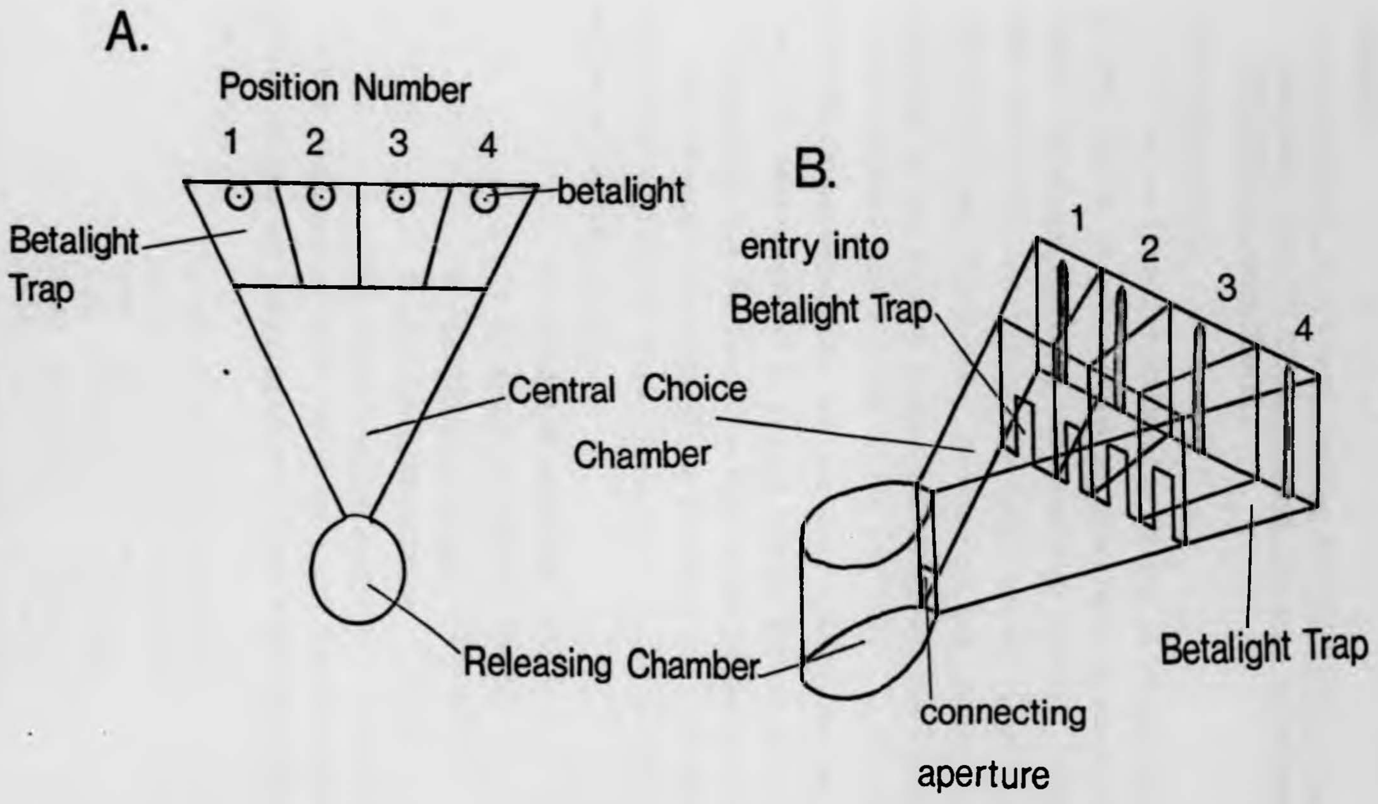


FIGURE 5. Wavelength Discrimination Chamber

RESULTS

1. Response of different species and different stages of Triatomid bug to betalights of various brightnesses and various wavelengths

(a) Introduction

In an earlier investigation it was possible to demonstrate a diel rhythm of attraction to betalight in adult male and female *Triatoma maculata* (Andrewartha, 1972). The work in that study was limited to two types of betalight, namely S10/G/2000 and S10/R/360. However it has since been possible to go on and extend the range of the investigation to incorporate experiments which not only utilise betalights of various brightnesses and various wavelengths but also to test the responses of different stages and different species of triatomid bug to these various kinds of betalight.

The results are summarized in Table 1. It can be seen from that table that five different species of Triatomid bug were tested, namely:

1. *Rhodnius prolixus* Stal.
2. *Triatoma infestans* (Klug)
3. *Triatoma maculata* (Erichson)
4. *Triatoma phyllosoma* (Stal)
5. *Panstrongylus megistus* (Burmeister)

The response of both adult male and female and different instar nymphs of these species to the following betalights was tested:

Green S10/G/2000 (sphere)
Green C135/G/280 (cylinder)
Red S10/R/360 (sphere)
Red C135/R/40 (cylinder)
Blue C135/B/90 (cylinder)
White C135/W/200 (cylinder)
Yellow C135/Y/280 (cylinder)

TABLE 1

Response of different stages and different species of Triatomid bug to betalights of various brightnesses and various wavelengths.

Species & stage	specification of betalight						
	Blue C135/B/90 10.5 x 0.7 cm Cylinder	Red C135/R/40 10.5 x 0.7 cm Cylinder	White C135/W/200 10.5 x 0.7 cm Cylinder	Green C135/G/280 10.5 x 0.7 cm Cylinder	Green S10/G/2000 Sphere	Red S10/R/360 Sphere	Yellow C135/Y/280 10.5 x 0.7 cm Cylinder
Rhodnius prolixus Adult ♂	Response Tested	Response Tested	Response Tested	Response Tested	Response Tested	Response Tested	Response Tested
Rhodnius prolixus Adult ♀	Response Tested	Response Tested	Response Tested	Response Tested	Response Tested	Response Tested	Response Tested
Rhodnius prolixus 5th	Response Tested	Response Tested	Response Tested		Response Tested		
Rhodnius prolixus 4th	Response Tested	Response Tested			Response Tested		Response Tested
Rhodnius prolixus 3rd					Response Tested	Response Tested	
Rhodnius prolixus 2nd	Response Tested						
Rhodnius prolixus 1st					Response Tested	Response Tested	

Species & stage	specification of betalight						
	Blue C135/B/90 10.5 x 0.7 cm Cylinder	Red C135/R/40 10.5 x 0.7 cm Cylinder	White C135/W/200 10.5 x 0.7 cm Cylinder	Green C135/G/280 10.5 x 0.7 cm Cylinder	Green S10/G/2000 Sphere	Red S10/R/360 Sphere	Yellow C135/Y/280 10.5 x 0.7 cm Cylinder
Triatoma infestans Adult ♀	Response Tested	Response Tested		Response Tested			Response Tested
Triatoma infestans 5th	Response Tested	Response Tested			Response Tested		Response Tested
Triatoma infestans 4th	Response Tested	Response Tested	Response Tested		Response Tested		Response Tested
Triatoma infestans 3rd	Response Tested	Response Tested			Response Tested		
Triatoma infestans 2nd				Response Tested		Response Tested	
Triatoma maculata Adult ♂	Response Tested	Response Tested		Response Tested	Response Tested	Response Tested	Response Tested
Triatoma maculata Adult ♀					Response Tested	Response Tested	
Triatoma maculata 4th			Response Tested		Response Tested		Response Tested
Triatoma maculata 3rd	Response Tested	Response Tested			Response Tested		
Triatoma maculata 2nd					Response Tested	Response Tested	
Triatoma phyllosoma 5th	Response Tested				Response Tested	Response Tested	Response Tested
Panstrongylus megistus Adult ♂	Response Tested			Response Tested	Response Tested		Response Tested

(b) Experimental Insectary

All experimental work in this investigation was carried out in an underground insectary in the Department of Entomology at the London School of Hygiene and Tropical Medicine. The ambient temperature within the insectary could be maintained at a fairly constant level thereby providing the required environmental conditions. Continuous thermohygrograph records were kept and periodically checked against a mercury thermometer and whirling hygrometer. The temperature in the insectary was generally maintained at 27.0 ± 5.0°C. The relative humidity was 80 ± 10%. The insectary also furnished reliable light proof conditions which were warranted in this investigation.

(c) Experimental Procedure

A full description of the apparatus used here was given previously. In studying the response of adult males and females, 5th instar nymphs, 4th instar nymphs, and 3rd instar nymphs of triatomid bugs to various types of betalight, the Polythene Choice Chamber was used. For 2nd and 1st instar nymphs the Glass Choice Chamber was used.

Before studying the phototactic responses of the bugs, all stages were kept in the Photoperiodic Entrainment Chamber for at least a week. In this way the onset and duration of the general activity of the bugs could be governed very precisely to suit the needs of the investigator. Generally, unless stated otherwise, the following photoperiod was adopted: a scotophase from 1300-1500 hours and a photophase from 1500-1300 hours. If the bugs were not being used for an immediate experiment they were kept in the Photoperiodic Entrainment Chamber.

The earlier investigation on Triatoma maculata (Andrewartha, 1972) had shown conclusively that only hungry bugs would show a reasonable response to betalight and so, where possible, bugs which had fed some weeks previously or had emerged some time previously but had not been fed as adults were used in the following experiments.

The experimental procedure was the same throughout. Using either the polythene choice chamber or the glass choice chamber the total number of bugs attracted to a betalight source over a 24-hour observation period was noted. At each hourly observation a record was made of the total number of bugs which had entered the betalight trap and the control trap (if any).

Each experiment was usually commenced at 0600 hours which was within the limits of the photophase that the bugs received in their photoperiodic entrainment. Since the bugs were subjected in their photoperiodic entrainment to light at 0600 hours it was possible to commence the experiment in an illuminated insectary. A total of 20 bugs (always 20, unless stated otherwise) was introduced into the central chamber of the polythene choice chamber via a perspex tube. The perspex tube reaches down to the floor of the central chamber so that on introducing the bugs they are not immediately released into the central chamber. The glass choice chamber also had a similar glass cylinder which effectively contained the bugs and prevented their release into the central chamber before the experiment was commenced.

The light in the insectary was then turned off and the insectary was maintained in total darkness throughout the run of the experiment. The perspex tube was removed from the central chamber, thus releasing the bugs. This marked the start of the experiment.

At hourly intervals the choice chamber was examined and the total number of bugs which had entered the betalight trap and control trap during that period was noted. This operation necessarily involved using the dim light of a small torch.

After noting the total number of bugs in each trap, any bugs which had fallen into the traps were returned to the chamber. Thus for each hour a total of 20 bugs (unless stated otherwise) was available to be attracted to betalight.

In analysing the data it was considered suitable to determine the total number of bugs which entered the betalight trap over a period of 3 hours. If 20 bugs are available in the chamber at each hourly interval, then for each 3-hour period a total of 60 bugs is available to be attracted to betalight. The percentage attraction to the betalight source for a 3-hour period can then be calculated and over a period of 24 hours the diel rhythm of attraction can be determined.

In an earlier study 24 hour observations were necessary since the bugs had been photoperiodically entrained with a scotophase from 1800-0900 hours and a photophase from 0900-1800 hours. The bugs were therefore active during the night hours and early morning and in order to gain some idea of the diel rhythm of attraction to the light source, repeated 24-hour observations, adopting the experimental procedure just described, were necessary. However with the more convenient photoperiodic entrainment it was possible to start the experiment at 0600 hours and finish observations at 2200 hours. Although bugs would not be active and thus not respond to the light source after 2100 hours, they were left in the apparatus overnight. The control trap and betalight trap could then be checked the following morning to see whether any bugs had entered the traps. In nearly all cases the traps were empty and hence it was possible to conclude that the bugs had not been active and not responded to the betalight.

(d) Results

The detailed results are shown in the following tables and a summary of these is presented in Table 1. The data indicate that in the five species studied here there is a phototactic response to betalights of varied microlambert values and different spectral composition. Tables 2-16 also indicate a distinct diel rhythm in the bugs' attraction to a betalight source. The diel rhythm of attraction to S10/G/2000 in 3rd instar nymphs of *Triatoma infestans* is shown in figure 6.

In general the environmental conditions in the

Polythene Choice Chamber in each experiment presented in Tables 2-16 approximated to the following:

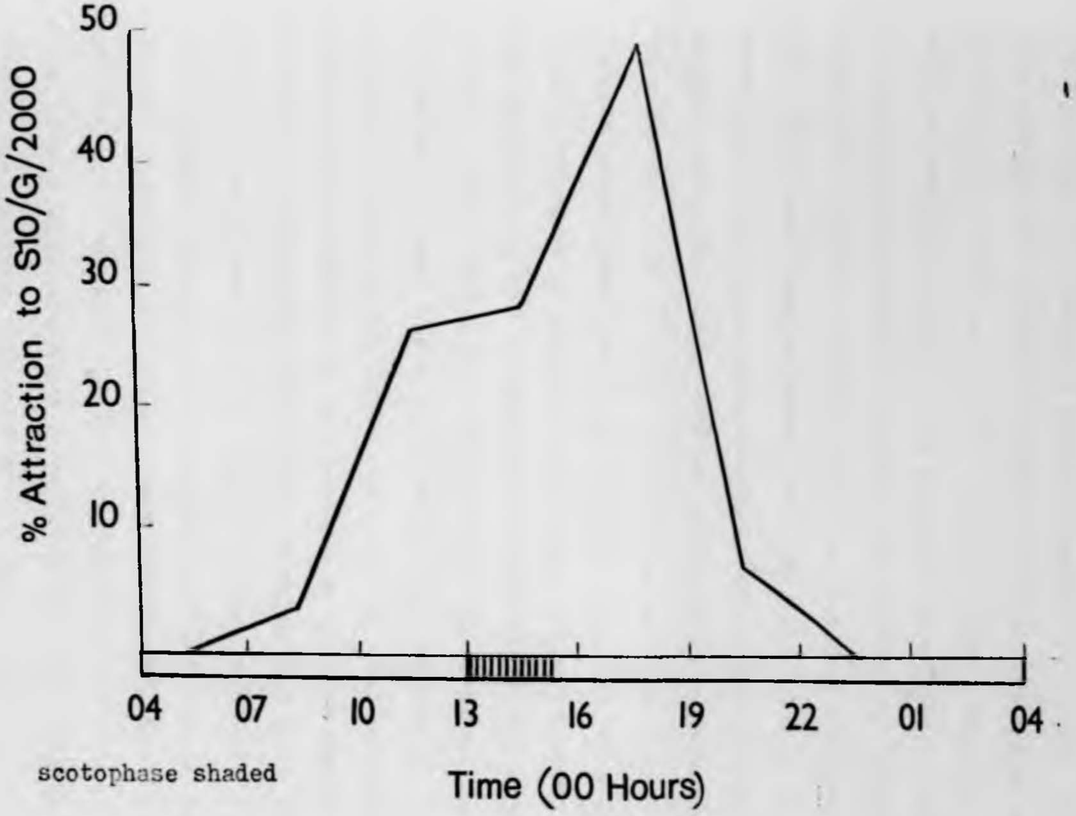
Temperature 26.9°C.
Relative humidity 70.0 ± 8.0%

An interesting feature emerges from the mass of data presented in Tables 2-16. It will be remembered that the results relate to percentage attraction to betalights of varied brightness and wavelength not only in different species and instars but also in an obviously diverse physiological group, e.g. bugs with a varied nutritional history. In all cases studied here the mean maximum percentage attraction to betalight was only 57.32%. This figure was obtained by checking each Table for the highest maximum percentage response to betalight and this yielded the following percentages:

66.6	64.7	70.1
50.0	45.5	35.8
65.0	53.3	49.1
70.1	68.0	55.4
50.0	55.0	61.0

A mean value was then calculated from these maxima.

The mean figure of 57.32% has particular relevance when considering the work of Kimmins (1971) who tested the phototactic responses of the Bark beetle. Kimmins, using a wavelength discrimination chamber similar to the one employed here (see figure 5), initially tested the wavelength sensitivity of 200 beetles and found that 53% of these entered a trap illuminated by ultraviolet light, 24% went to one of the other 9 traps, and 23% either moved back into the dark releasing chamber



scotophase shaded

Figure 6. Diel rhythm of attraction of 3rd instar nymphs of *Triatoma infestans* to green betalight, S10/G/2000 shown in relation to previous photoperiodic entrainment.

or out into the central choice chamber or arena. Kimmins then tested the group of bugs which had not shown any response to light and found that the same response pattern emerged, namely 47% to the ultraviolet, 35% to other wavelengths of light, 18% showing no response. In re-testing the beetles which had shown a response to the other wavelengths there was then found to be a consistent total group response to the ultra-violet light source, i.e. 48%, 49%, 57% and 55% in four consecutive experiments. It is also interesting to note that Kimmins never obtained a 100% total response or a 100% total avoidance of ultra-violet even after re-testing the group which had originally responded to the ultra-violet. From this data, Kimmins therefore suggests, tentatively, that there is a relatively constant probability in the insect's response to ultra-violet, although he presents no conclusions as to the actual behavioural mechanism involved. Perhaps the mean figure of 57.32% obtained in this study in some way fits in with Kimmins' theory of a constant probability in the insect's response to light. Furthermore the fact that Kimmins never obtained a 100% response in all his experiments suggests that in a laboratory situation, at least, the maximum response to a light source will never exceed a value of approximately 70%, bearing in mind that 70-75% was the maximum total response obtained in this study. It would indeed be interesting to know whether the constant probability of response to a light source, i.e. in the range of 50-70%, has significance in the field. If so, it will need to be considered as an integral factor likely to influence light trap catches.

Finally, the question of sensory adaptation to the light stimulus will be considered in the Discussion in the context of other experiments carried out in this investigation.

TABLE 2

Percentage Attraction to Betalights of different brightnesses and different wavelengths

Rhodnius prolixus Adult males

Time	Green cylinder C135/G/280	Blue cylinder C135/B/90	White cylinder C135/W/200	Red cylinder C135/R/40
0700-1000	5.0	3.3	5.0	1.6
1000-1300	33.3	31.6	40.0	18.3
1300-1600	30.0	31.6	26.6	20.0
1600-1900	28.3	20.0	16.6	16.6
1900-2200	5.0	8.3	5.0	5.0
2200-0100	0.0	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

	Green sphere S10/G/2000	Yellow cylinder C135/Y/280	Red sphere S10/R/360
0700-1000	3.0	5.2	2.4
1000-1300	16.6	29.8	21.4
1300-1600	66.6	22.8	35.7
1600-1900	50.0	15.7	16.6
1900-2200	30.0	1.7	2.4
2200-0100	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0

TABLE 3

Percentage Attraction to Betalights of different brightnesses and different wavelengths

Rhodnius prolixus Adult females

Time	Blue cylinder C135/B/90	Green sphere S10/G/2000	Yellow cylinder C135/Y/280	Red cylinder C135/R/40
0700-1000	10.0	7.4	1.9	3.3
1000-1300	43.3	56.6	64.7	40.0
1300-1600	46.6	63.3	52.9	28.3
1600-1900	30.0	26.6	43.1	20.0
1900-2200	1.0	3.0	9.8	5.0
2200-0100	0.0	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

	Green cylinder C135/G/280	White cylinder C135/W/200	Red sphere S10/R/360
0700-1000	5.0	3.9	2.2
1000-1300	13.3	21.5	8.8
1300-1600	30.0	23.7	20.0
1600-1900	26.6	13.7	46.6
1900-2200	1.6	1.9	4.4
2200-0100	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0

TABLE 4.

Percentage Attraction to Betalights of different brightnesses and different wavelengths

Rhodnius prolixus 5th instar nymphs

	Blue cylinder C135/B/90		Green sphere S10/G/2000		Red cylinder C135/R/40		White cylinder C135/W/200	
Time	B.T.	C.T.	B.T.	C.T.	B.T.	C.T.	B.T.	C.T.
0700-1000	1	0	4	0	0	0	0	0
1000-1300	18	2	36	4	8	3	7	0
1300-1600	34	5	61	5	21	4	12	0
1600-1900	20	7	51	7	29	3	18	1
1900-2200	2	0	24	3	8	3	3	0
2200-0100	0	0	0	0	0	0	0	0
0100-0400	0	0	0	0	0	0	0	0
0400-0700	0	0	0	0	0	0	0	0
Totals	75	14	176	19	66	13	40	1

	Percentage Attraction	Percentage Attraction	Percentage Attraction	Percentage Attraction
0700-1000	1.0	4.5	0.0	0.0
1000-1300	19.3	41.3	14.3	12.2
1300-1600	36.5	70.1	36.8	21.5
1600-1900	21.4	57.6	50.9	31.5
1900-2200	2.1	27.5	14.3	5.2
2200-0100	0.0	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

Abbreviations: B.T. = Betalight Trap
C.T. = Control Trap

TABLE 5

Percentage Attraction to betalights of different brightnesses and different wavelengths

Rhodnius prolixus 4th instar nymphs

Time	Red cylinder C135/R/40	Yellow cylinder C135/Y/280	Blue cylinder C135/B/90	Green sphere S10/G/2000
0700-1000	1.6	3.3	1.6	3.3
1000-1300	21.6	13.3	20.0	15.0
1300-1600	30.0	21.6	20.0	20.0
1600-1900	10.0	8.3	25.0	15.0
1900-2200	5.0	3.3	1.6	1.6
2200-0100	0.0	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

Rhodnius prolixus 3rd instar nymphs

Time	Green sphere S10/G/2000	Red sphere S10/R/360
0700-1000	15.4	4.1
1000-1300	50.0	20.8
1300-1600	40.4	25.0
1600-1900	33.3	4.1
1900-2200	0.0	0.0
2200-0100	0.0	0.0
0100-0400	0.0	0.0
0400-0700	0.0	0.0

TABLE 6

Percentage Attraction to Betalights of different brightnesses and different wavelengths

Rhodnius prolixus 2nd instar nymphs

Time	Blue cylinder C135/B/90
0700-1000	0.0
1000-1300	22.0
1300-1600	23.3
1600-1900	18.6
1900-2200	6.0
2200-0100	1.3
0100-0400	0.0
0400-0700	0.0

Rhodnius prolixus 1st instar nymphs

Time	Green sphere S10/G/2000	Red sphere S10/R/360
0700-1000	9.5	5.3
1000-1300	45.5	33.3
1300-1600	32.6	23.3
1600-1900	17.0	11.3
1900-2200	2.7	1.3
2200-0100	0.0	0.0
0100-0400	0.0	0.0
0400-0700	0.0	0.0

TABLE 7

Percentage Attraction to Betalights of different brightnesses and different wavelengths

Triatoma infestans Adult females

Time	Blue cylinder C135/B/90	Yellow cylinder C135/Y/280	Red cylinder C135/R/40	Green cylinder C135/G/280
0700-1000	5.1	5.1	3.3	3.3
1000-1300	23.0	30.7	20.0	16.6
1300-1600	35.8	28.1	26.6	16.6
1600-1900	23.0	12.8	13.3	10.0
1900-2200	2.5	5.1	3.3	3.3
2200-0100	0.0	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

TABLE 8

Percentage Attraction to betalights of different brightnesses and different wavelengths

Triatoma infestans 5th instar nymphs

Time	Blue cylinder C135/B/90	Yellow cylinder C135/Y/280	Green sphere S10/G/2000	Red cylinder C135/R/40
0700-1000	3.3	1.6	1.6	0.0
1000-1300	40.0	28.3	36.6	8.3
1300-1600	41.6	31.6	65.0	16.6
1600-1900	21.6	30.0	18.3	23.3
1900-2200	3.3	3.3	1.6	5.0
2200-0100	0.0	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

TABLE 9

Percentage Attraction to betalights of different brightnesses and different wavelengths

Triatoma infestans 4th instar nymphs

Time	Blue cylinder C135/B/90	Red cylinder C135/R/40	White cylinder C135/W/200	Yellow cylinder C135/Y/280
0700-1000	2.2	0.0	2.2	0.0
1000-1300	13.3	4.4	20.0	13.3
1300-1600	46.6	15.5	53.3	42.2
1600-1900	35.5	8.8	33.3	31.1
1900-2200	15.5	2.2	4.4	2.2
2200-0100	4.4	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

	Green sphere S10/G/2000
0700-1000	3.3
1000-1300	48.3
1300-1600	35.0
1600-1900	28.3
1900-2200	11.6
2200-0100	0.0
0100-0400	0.0
0400-0700	0.0

TABLE 10

Percentage Attraction to Betalights of different brightnesses and different wavelengths

Triatoma infestans 3rd instar nymphs

TIME	Green Sphere S10/G/2000	Blue Cylinder C135/B/90	Red Cylinder C135/R/40
0700-1000	3.5	1.6	0.0
1000-1300	26.3	18.3	8.3
1300-1600	28.7	20.0	15.0
1600-1900	49.1	33.3	11.6
1900-2200	7.0	1.6	3.3
2200-0100	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0

Triatoma infestans 2nd instar nymphs

TIME	Red Sphere S10/R/360	Green Cylinder C135/G/280
0700-1000	5.0	3.3
1000-1300	46.6	40.0
1300-1600	25.0	28.3
1600-1900	26.6	33.3
1900-2200	8.3	11.6
2200-0100	0.0	0.0
0100-0400	0.0	0.0
0400-0700	0.0	0.0

TABLE 11

Percentage Attraction to Betalights of different brightnesses and different wavelengths

Triatoma maculata Adult males

Time	Green Sphere S10/G/2000	Red Sphere S10/R/360	Blue Cylinder C135/B/90	Red Cylinder C135/R/40
0700-1000	7.8	3.0	8.4	3.3
1000-1300	70.1	30.3	50.4	39.8
1300-1600	42.8	16.7	39.3	27.2
1600-1900	15.4	7.8	20.0	19.0
1900-2200	3.4	2.0	1.4	4.2
2200-0100	0.0	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

Time	Yellow Cylinder C135/Y/280	Green Cylinder C135/G/280
0700-1000	1.2	4.9
1000-1300	60.2	15.3
1300-1600	51.0	35.5
1600-1900	39.7	28.2
1900-2200	10.0	2.0
2200-0100	0.0	0.0
0100-0400	0.0	0.0
0400-0700	0.0	0.0

TABLE 12

Percentage Attraction to betalights of different brightnesses and different wavelengths

Triatoma maculata adult females

Time	Green sphere S10/G/2000	Red sphere S10/R/360
0700-1000	0.0	0.0
1000-1300	14.2	13.0
1300-1600	68.0	25.1
1600-1900	26.6	16.0
1900-2200	13.0	6.4
2200-0100	4.0	5.0
0100-0400	0.0	0.0
0400-0700	0.0	0.0

TABLE 13

Percentage Attraction to betalights of different brightnesses and different wavelengths

Triatoma maculata 4th instar nymphs

Time	White cylinder C135/W/200	Yellow cylinder C135/Y/280	Green sphere S10/G/2000
0700-1000	2.2	2.2	0.0
1000-1300	20.0	13.3	23.4
1300-1600	51.1	51.1	55.4
1600-1900	28.8	24.4	15.8
1900-2200	4.4	2.2	6.2
2200-0100	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0

TABLE 14

Percentage Attraction to betalights of different brightnesses and different wavelengths

Triatoma maculata 3rd instar nymphs

Time	Green sphere S10/G/2000	Blue cylinder C135/B/90	Red cylinder C135/R/40
0700-1000	3.9	8.3	5.0
1000-1300	17.6	33.3	31.6
1300-1600	49.0	30.0	26.6
1600-1900	29.4	13.3	26.6
1900-2200	3.9	6.6	13.3
2200-0100	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0

Triatoma maculata 2nd instar nymphs

Time	Green sphere S10/G/2000	Red sphere S10/R/360
0700-1000	5.0	1.3
1000-1300	50.0	18.0
1300-1600	25.0	13.4
1600-1900	16.6	6.5
1900-2200	5.0	1.3
2200-0100	0.0	0.0
0100-0400	0.0	0.0
0400-0700	0.0	0.0

TABLE 15

Percentage Attraction to Betalights of different brightnesses and different wavelengths

Triatoma phyllosoma 5th instar nymphs

Time	Green Sphere S10/G/2000	Blue Cylinder C135/B/90	Yellow Cylinder C135/Y/280	Red Sphere S10/R/360
0700-1000	1.6	0.0	1.6	0.0
1000-1300	30.0	33.3	38.3	13.3
1300-1600	46.6	55.0	51.6	28.3
1600-1900	20.0	23.3	20.0	16.6
1900-2200	8.3	10.0	10.0	11.6
2200-0100	1.6	1.6	1.6	3.3
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

TABLE 16

Percentage Attraction to Betalights of different brightnesses and different wavelengths

Panstrongylus megistus Adult males

Time	Green Sphere S10/G/2000	Blue Cylinder C135/B/90	Green Cylinder C135/G/280	Yellow Cylinder C135/Y/280
0700-1000	3.3	6.6	3.3	3.3
1000-1300	26.6	31.6	40.0	35.0
1300-1600	58.3	61.6	55.0	50.0
1600-1900	48.3	43.3	48.3	26.6
1900-2200	25.0	28.3	16.6	35.0
2200-0100	3.3	0.0	1.6	1.6
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

2. Brightness as a factor determining the magnitude of response to a betalight source

(i) Introduction

Betalights are not only manufactured in a variety of different colours but also a range of different brightnesses. As stated previously the brightness of any given betalight is determined by the amount of contained tritium. The brightness of a betalight is given in microlamberts but approximate conversion factors to other units are given below:

$$\begin{aligned} 1 \text{ lambert} &= \frac{1}{\pi \; 10^{-4}} \text{ nits} \\ &= 296 \text{ cd ft}^{-2} \\ &= 929 \text{ foot-lamberts} \\ &= \frac{1}{\pi} \text{ stilb} \\ &= 10^{4} \text{ apostilb} \end{aligned}$$

It is important to note that all brightnesses as quoted in the technical literature provided by the manufacturing company are average values and are subject, in manufacture, to variations which are usually within ± 15%. It is also important to remember that the microlambert units quoted in the technical literature are estimated on the basis of human optic sensitivity. This does not therefore necessarily equate to the brightnesses as seen by other animals, in this case triatomid bugs.

Nevertheless, in the experiments described below it will be seen that the brightness of the betalight source is an important factor and will determine the magnitude of the bug's response. Even though the brightnesses of the betalights used here have been measured according to the sensitivity of the human eye, this factor still appears to have relevance for triatomid bugs since betalights of higher microlambert values attract a significantly larger number of bugs. This does indicate that arthropods can differentiate varied intensities of light measured on the microlambert scale.

(ii) The sensitivity of 1st instar nymphs of *Rhodnius prolixus* to green betalights of different brightnesses

The responses of 1st instar nymphs of *Rhodnius prolixus* to betalight were tested in a glass choice chamber. The choice chamber was equipped with two lights, a green spherical betalight of 2000 microlamberts at one end, and, simultaneously, at the other a green strip betalight of 280 microlamberts. The strip betalight, C135/G/280, was taped over with black adhesive so that the total surface area of light presented by the two betalights was exactly the same.

After hatching from the egg, the nymphs were starved for a period of 25 days before running the experiment. The bugs' responses to the two betalights were observed over a period of 24 hours by noting the total number of bugs falling into the two traps at either end of the glass choice chamber. The experiment was carried out on three occasions, each experiment consisting of twenty bugs.

The temperature and relative humidity within the choice chamber could not be measured and so it must be assumed that these two environmental factors approximated to the ambient of the insectary.

The results are presented in Table 17. The stronger brightness attracted a mean catch of 55% at a peak between 1300 hours and 1600 hours, trapping before and after the peak being less at all times. The range of attraction in the three experiments for the peak period was 50% to 60%. The betalight of lesser brightness, 280 microlamberts, always yielded a lower catch. The catch attained a peak of 19.9% between 1300 hours and 1600 hours, the replicate range being 18.3% to 21.6%.

TABLE 17

The sensitivity of 1st instar nymphs of R. prolixus to green betalights of different brightnesses

Time	Percentage attraction to S10/G/2000			Mean	Percentage attraction to C135/G/280			Mean
0700-1000	3.3	1.6	5.0	3.3	0	1.6	1.6	1.1
1000-1300	43.3	48.3	48.3	46.6	21.6	20.0	15.0	18.8
1300-1600	50.0	60.0	55.0	55.0	20.0	18.3	21.6	19.9
1600-1900	10.0	13.3	13.3	12.2	6.6	5.0	8.3	6.6
1900-2200	3.3	3.3	3.3	3.3	1.6	0	1.6	1.1
2200-0100	0	0	0	0	0	0	0	0
0100-0400	0	0	0	0	0	0	0	0
0400-0700	0	0	0	0	0	0	0	0

(iii) The influence of the factor of brightness in the response of 3rd instar nymphs of Triatoma maculata to green betalight

The responses of 3rd instar nymphs of Triatoma maculata to betalight were tested in a polythene choice chamber. The bugs, as before, had been subjected to a photoperiodic entrainment with a scotophase from 1300 hours to 1500 hours. The bugs had been post-emergence starved for 26 days. The experimental procedure outlined above was adopted here. The results are tabulated below.

TABLE 18

The sensitivity of 3rd instar nymphs of *T. maculata* to green betalights of different brightnesses

Time	Percentage attraction to S10/G/2000			Mean	Percentage attraction to C135/G/280			Mean
0700-1000	1.6	0	3.3	1.6	1.6	3.3	1.6	2.1
1000-1300	40.0	40.0	41.6	40.5	18.3	20.0	21.6	19.9
1300-1600	50.0	53.3	50.0	51.1	26.6	25.0	26.6	26.0
1600-1900	11.6	13.3	13.3	12.7	6.6	8.3	5.0	6.6
1900-2200	3.3	5.0	6.6	4.9	1.6	3.3	1.6	2.1
2200-0100	0	0	0	0	0	0	0	0
0100-0400	0	0	0	0	0	0	0	0
0400-0700	0	0	0	0	0	0	0	0
Total 24 hour catch	64	67	69		33	36	34	

A similar result was obtained for 3rd instar *T. maculata*. The catch in the 2000 microlambert betalight trap ranged from 40% to 53.3% between 1000 hours and 1600 hours, whereas over the same period a catch ranging from only 18.3% to 26.6% was achieved with the 280 microlambert green betalight.

(iv) The sensitivity of adult females of *Triatoma infestans* to green betalights of different brightnesses

A polythene choice chamber was used to test the phototactic responses of adult female *Triatoma infestans*. The experimental procedure adopted in the previous experiments was followed here. The bugs were post-emergence starved for 25 days. The results are shown in Table 19. The results are shown graphically in Figure 7. The stronger brightness (2000 microlamberts) attracted a mean catch of 51.6% at a peak between 1600 and 1900 hours, the experimental procedure outlined above was adopted here. The results are tabulated below.

TABLE 18

The sensitivity of 3rd instar nymphs of *T. maculata* to green betalights of different brightnesses

Time	Percentage attraction to S10/G/2000			Mean	Percentage attraction to C135/G/280			Mean
0700-1000	1.6	0	3.3	1.6	1.6	3.3	1.6	2.1
1000-1300	40.0	40.0	41.6	40.5	18.3	20.0	21.6	19.9
1300-1600	50.0	53.3	50.0	51.1	26.6	25.0	26.6	26.0
1600-1900	11.6	13.3	13.3	12.7	6.6	8.3	5.0	6.6
1900-2200	3.3	5.0	6.6	4.9	1.6	3.3	1.6	2.1
2200-0100	0	0	0	0	0	0	0	0
0100-0400	0	0	0	0	0	0	0	0
0400-0700	0	0	0	0	0	0	0	0
Total 24 hour catch	64	67	69		33	36	34	

A similar result was obtained for 3rd instar *T. maculata*. The catch in the 2000 microlambert betalight trap ranged from 40% to 53.3% between 1000 hours and 1600 hours, whereas over the same period a catch ranging from only 18.3% to 26.6% was achieved with the 280 microlambert green betalight.

(iv) The sensitivity of adult females of Triatoma infestans to green betalights of different brightnesses

A polythene choice chamber was used to test the phototactic responses of adult female *Triatoma infestans*. The experimental procedure adopted in the previous experiments was followed here. The bugs were post-emergence starved for 25 days. The results are shown in Table 19. The results are shown graphically in Figure 7. The stronger brightness (2000 microlamberts) attracted a mean catch of 51.6% at a peak between 1600 and 1900 hours, the range of attraction in the replicates being 50% to 53.3%. The weaker brightness (280 microlamberts) yielded a peak catch of 23.8%, the replicate range being 21.6% to 25%.

TABLE 19

The sensitivity of adult females of T. infestans to green betalights of different brightnesses

Time	Percentage attraction to S10/G/2000			Mean	Percentage attraction to C135/G/280			Mean
0700-1000	3.3	5.0	5.0	4.4	3.3	5.0	5.0	4.4
1000-1300	26.6	28.3	30.0	28.3	3.3	6.6	5.0	4.9
1300-1600	40.0	40.0	43.3	41.1	15.0	16.6	16.6	16.0
1600-1900	51.6	50.0	53.3	51.6	21.6	25.0	25.0	23.8
1900-2200	26.6	28.3	31.6	28.8	18.3	20.0	23.3	20.5
2200-0100	0	0	0	0	0	1.6	3.3	1.6
0100-0400	0	0	0	0	0	0	0	0
0400-0700	0	0	0	0	0	0	0	0
Total 24 hour catch	89	91	98		37	45	47	

The results indicate the greater brightness of betalight, in the range tested, increased the phototactic responses of bugs. Hinde (1966) has explained the greater response by organisms to stimuli of high intensity on the basis of two physiological observations. First, a higher stimulus intensity results in the excitation of a higher proportion of the fibres leaving the sense organ. Second, there appears to be a relationship between stimulus intensity and nerve impulse frequency.

It is suggested that no matter what colour is used in the betalight range, a betalight of higher microlambert value is recommended for use in the field. This generalization might hold true not only for different species but also different instars.

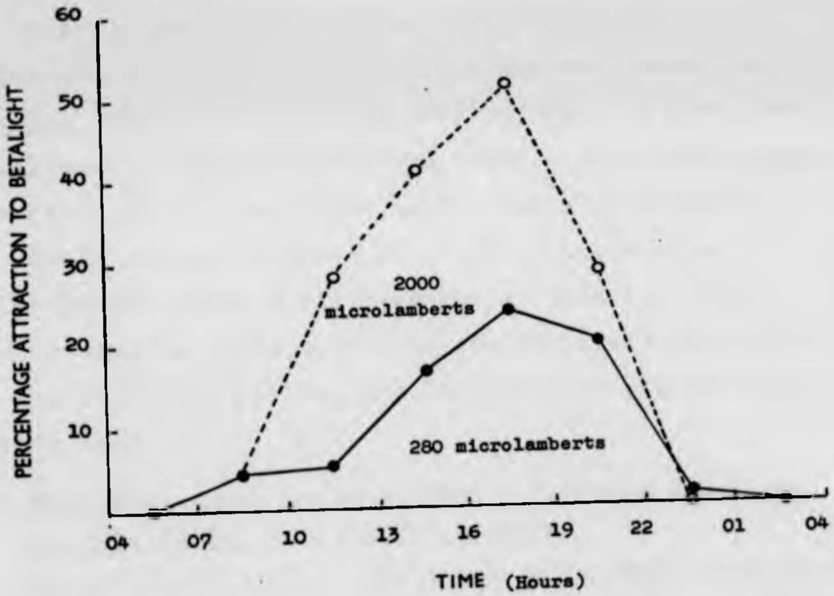


Figure 7. The response of adult female T. infestans to green betalights of 280 and 2000 microlamberts.

3. The sensitivity of triatomid bugs to betalights of different colours

(i) Introduction

The importance of brightness in determining the levels of phototactic response has already been studied and the aim of the following experiments is to assess how important colour sensitivity might be in the response of triatomid bugs to a betalight source. Investigations of spectral sensitivity can be quite complex, for example Snow's study (1971) of the spectral sensitivity of *Aedes aegypti* (L.) at oviposition, and, as such, can constitute a study in their own right. The investigation presented here was no more than a simple assessment of the way in which different coloured betalights can influence the phototactic response of triatomid bugs. Further studies along this line are certainly warranted and the following preliminary findings are presented as a guide line.

(ii) The sensitivity of adult female *Triatoma infestans* to betalights of different colours

Colour sensitivity in adult female *Triatoma infestans* was studied using the wavelength discrimination chamber described previously. The four betalight traps in the chamber were each equipped with a betalight of a different wavelength or colour. The following betalights were used:

Serial number	Colour	Brightness in microlamberts
C135/G/280	Green	280
C135/Y/280	Yellow	280
C135/R/40	Red	40
C135/B/90	Blue	90

The following dimensions apply to each of the betalights used in this study. All cylinders had an overall length of 105 mm. The actual illuminated length equalled 99 mm. The diameter and bore of each cylinder equalled 7.25 mm. and 5.0 mm. respectively.

It will be seen from the information above that the betalights used were not of equal microlambert values. Ideally, all betalights should have been of 280 microlamberts. This difference in microlambert value prevents a proper analysis of spectral sensitivity in triatomid bugs. It is not possible, unfortunately, to obtain specially made betalights from the manufacturers to suit the requirements of a spectral sensitivity study. Furthermore, as mentioned previously, levels of brightness in betalights are subject to inevitable variations in manufacture. On that basis therefore, the betalights used here had to suffice and the results obtained are discussed in the context of these anomalies.

The experimental procedure was essentially simple and consisted of noting the total number of bugs entering the four traps at each hour. Twenty adult female Triatoma infestans were placed in the releasing chamber. After release, the number of bugs in each trap was noted for the hour, and the twenty bugs were then returned to the releasing chamber. A total of twenty bugs was available to be attracted to each betalight at each hourly interval. The phototactic behaviour of the twenty bugs was studied over a 16 hour period, the experiment started at 0800 hours and completed at 2300 hours.

The bugs had been fed 22 days before the first experiment was run and had been subjected to a scotophase from 1300 hours to 1500 hours in their photoperiodic entrainment.

In order to obviate any bias on the part of any trap in the chamber, the position of each betalight was changed at each hour so that, for example, the red betalight, C135/R/40, would be positioned to occupy trap numbers 2, 1, 4 and 3 at each four hour observation period. The change in betalight position over a four hour period is shown below:

		trap number 1	trap number 2	trap number 3	trap number 4
Hours	1	green	red	yellow	blue
	2	red	yellow	blue	green
	3	yellow	blue	green	red
	4	blue	green	red	yellow

This procedure was adopted in the three subsequent four hour periods.

The experiment was run over a period of 5 days during which time the environmental conditions in the wavelength discrimination chamber approximated to the following: temperature 26.9°C. and relative humidity 70%.

The results are shown in Table 20 and Figure 8. The total number of bugs responding to each betalight was noted after each 16 hour experiment and a mean value was obtained from the five experiments, i.e. 53.2 (range 51 to 58) for C135/G/280, 13.2 (range 11 to 17) for C135/R/40, 18.0 (range 17 to 20) for C135/Y/280, and 66.0 (range 63 to 75) for C135/B/90.

A comparative ratio of response was calculated for each betalight type on the basis of these mean values:

Betalight serial number	Response expressed as a ratio
C135/B/90	1.000
C135/G/280	0.806
C135/Y/280	0.272
C135/R/40	0.200

There is a clear indication from these results that colour may indeed be an important factor likely to influence the attraction of triatomid bugs to betalight traps. There is, on the basis of the five experiments, a greater sensitivity to the blue betalight (90 microlamberts), decreasing from green (280 microlamberts) to yellow (280 microlamberts) to red (40 microlamberts). It is not possible to state categorically that triatomid bugs do in fact possess the ability to discriminate between different wavelength bands and that they therefore manifest some level of spectral sensitivity. This will only be possible if and when a more precise evaluation of wavelength discrimination is achieved with betalights of equal microlambert values and equal energy emission.

The results obtained here are nevertheless interesting. On the basis of microlambert values, the green and yellow betalights, both of 280 microlamberts, should theoretically have been considerably more attractive than the blue strip betalight of lower brightness. In fact, the blue strip betalight is virtually ⅓ rd of the microlambert value of both the green and the yellow betalight. Despite this the blue betalight attracted a considerably greater number of bugs, which, in itself, suggests some ability to distinguish or discriminate between different colours or wavelengths.

The results, furthermore, agree with the work of other investigators who have shown a greater sensitivity of insects to the shorter wavelengths of radiation. The red betalight, emitting longer wavelengths of radiation, proved to be the least attractive, although it must be remembered that of the four betalights it had the lowest brightness level.

Interestingly, Weiss (1946) has stated that "insects will react positively to all wavelengths from approximately 3650 Å to 7200 Å, the shorter wavelengths usually requiring much less intensity than the longer ones in bringing about a positive response." Weiss claims that this results from a greater sensitivity of the eyes of insects to the shorter wavelengths of radiation. Triatomid bugs, as shown in this study, also appear to be sensitive to the wavelength range designated by Weiss (1946) and it may be that the least attractive colours in the betalight range could be made more attractive by suitably increasing their brightnesses, as in the case of other insect groups (Weiss et al., 1942). It has not been shown in this investigation by how much the brightness would have to be increased, but this could certainly be considered in field studies of trapping.

A further consideration of the results is presented in the Discussion.

TABLE 20

Wavelength discrimination in adult female

Triatoma infestans

Time	Green					Red					Yellow					Blue				
	C135/G/280					C135/R/40					C135/Y/280					C135/B/90				
0800	1	3	3	1	0	0	1	1	1	0	0	1	0	0	0	0	0	2	1	1
0900	1	4	0	5	2	1	0	0	0	1	0	2	1	1	1	2	1	4	2	2
1000	3	0	2	0	2	1	1	2	1	1	2	1	0	4	3	1	1	0	2	0
1100	4	1	3	2	5	0	1	1	2	0	2	0	4	0	1	3	2	3	3	1
1200	4	4	3	3	4	4	1	1	0	2	4	3	0	0	2	7	6	5	5	5
1300	4	3	4	4	6	0	3	0	1	1	4	0	3	0	3	2	4	8	5	5
1400	2	4	4	6	3	1	2	0	2	1	0	1	1	3	1	8	6	7	6	7
1500	4	3	5	4	4	1	0	2	0	1	0	4	4	3	1	8	8	8	6	7
1600	3	3	8	9	3	2	3	2	2	2	1	2	1	3	1	8	7	6	4	9
1700	8	9	3	1	9	1	0	1	0	2	2	1	4	2	1	7	6	7	8	4
1800	3	5	3	8	4	1	2	1	2	0	1	1	1	1	1	7	6	8	6	8
1900	5	6	5	5	4	0	1	0	1	0	1	1	0	0	1	6	7	6	8	5
2000	4	1	1	2	4	0	1	0	1	0	1	0	0	0	0	3	3	4	2	4
2100	4	3	3	4	3	0	0	0	1	0	0	1	1	0	1	2	6	0	2	3
2200	1	0	3	3	2	0	1	0	0	0	0	0	0	0	0	1	0	6	2	3
2300	0	2	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0
Totals	51	51	51	58	55	12	17	11	14	12	18	18	20	17	17	65	63	75	63	64
Mean	53.2					13.2					18.0					66.0				

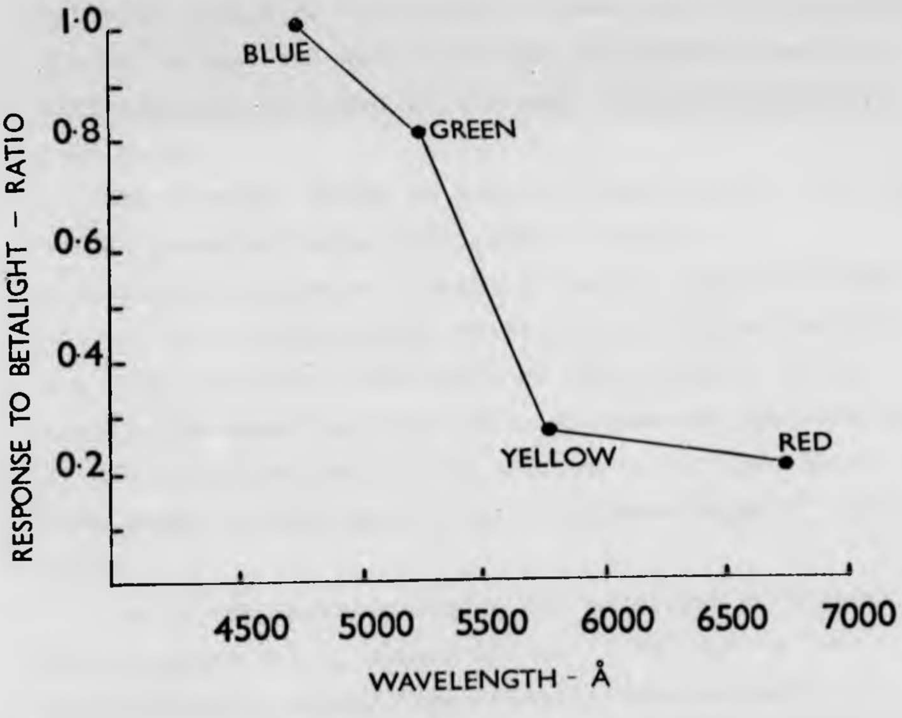


Figure 8. Wavelength discrimination in adult female *Triatoma infestans*

(iii) The sensitivity of adult female *Triatoma maculata* to red and green betalights

In this experiment the polythene choice chamber was used. One trap in the chamber was equipped with a red betalight of 360 microlamberts while the other, at the opposite end, was fitted with a green betalight of 280 microlamberts. The circadian rhythm of attraction to the two betalights was studied in 20 adult female *Triatoma maculata*. The bugs had been fed 20 days before the experiment and were entrained to manifest maximum activity over a period of 9 hours, from 1000 hours to 1900 hours.

The results, shown in Table 21 and Figure 9, are mean values based on three replicates. There is a significantly greater attraction to the green betalight of only 280 microlamberts which yielded a mean catch in the peak period of 1600 hours to 1900 hours of 53.3% (replicate range 46.6% to 60%), whereas the red betalight of 360 microlamberts in the peak period of 1300 hours to 1600 hours yielded only 36.6% (replicate range 33.3% to 40%).

As in the previous study, the betalight of higher microlambert value, namely the red betalight of 360 microlamberts, should theoretically have attracted a larger number of bugs. However, adult *Triatoma maculata* also manifest a greater sensitivity to a betalight source emitting shorter wavelengths of radiation. It was stated earlier that by increasing the brightness of a betalight of less attractive colour, the phototactic responses of bugs could be enhanced.

It may be argued that in this study the red betalight was not of a sufficiently high brightness to override the greater sensitivity to the green coloured betalight.

These two preliminary experiments have indicated some degree of colour sensitivity in two species of Triatoma. This phenomenon may apply equally to other species and so the colour of a light source should be given consideration in field studies.

TABLE 21

Colour sensitivity of adult Triatoma maculata

Mean percentage attraction to betalight

Time	S10/R/360 red	C135/G/280 green
0700-1000	1.6	3.3
1000-1300	10.0	13.3
1300-1600	36.6	43.3
1600-1900	23.3	53.3
1900-2200	16.6	30.0
2200-0100	0	0
0100-0400	0	0
0400-0700	0	0

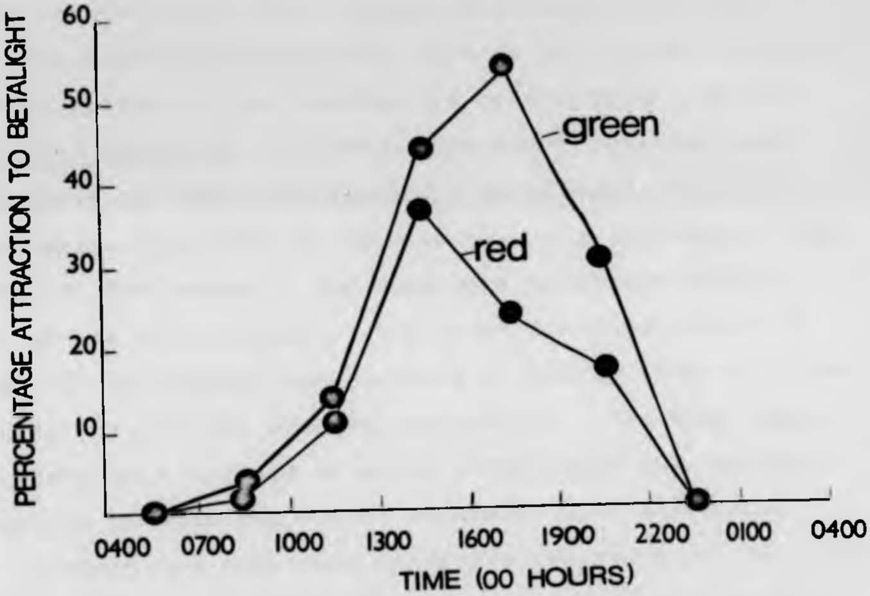


Figure 9. Wavelength Discrimination in Adult female <u>Triatoma maculata</u>

4. Distribution Studies and the phenomenon of Thigmotaxis

It was decided to study the distribution of bugs in the central chamber over an observation period of 24 hours. A suitable reference grid as shown in figure 10 was therefore mapped out on the floor of the chamber. Squares on the grid were marked thus: squares horizontally were marked 1 to 15 and squares vertically A to K.

The experiment described here is part of an earlier investigation of the phototactic behaviour of female Triatoma maculata. In this experiment the bugs were subjected in their photoperiodic entrainment to a scotophase from 1700 to 0900 hours and a photophase from 0900 to 1700 hours. The bugs were therefore active during the night hours. The exact distribution of 10 bugs in the chamber was recorded at hourly intervals over a complete 24 hour observation period. The bugs were observed with the aid of a dim torch light and the exact location of each bug on the reference grid was noted.

Clearly the bugs must orientate according to the light source glowing continuously from one end of the chamber. Theoretically, during the period corresponding to the photophase of the bugs' photoperiodic entrainment, i.e. their period of inactivity, there should be a distinct accumulation of bugs in the darker areas of the chamber, furthest away from the light source. To demonstrate this the reference grid was divided into two sections, one section including squares 1-8, the second squares 9-15. By observing the total number of bugs in each of the two sections it was therefore possible to determine the

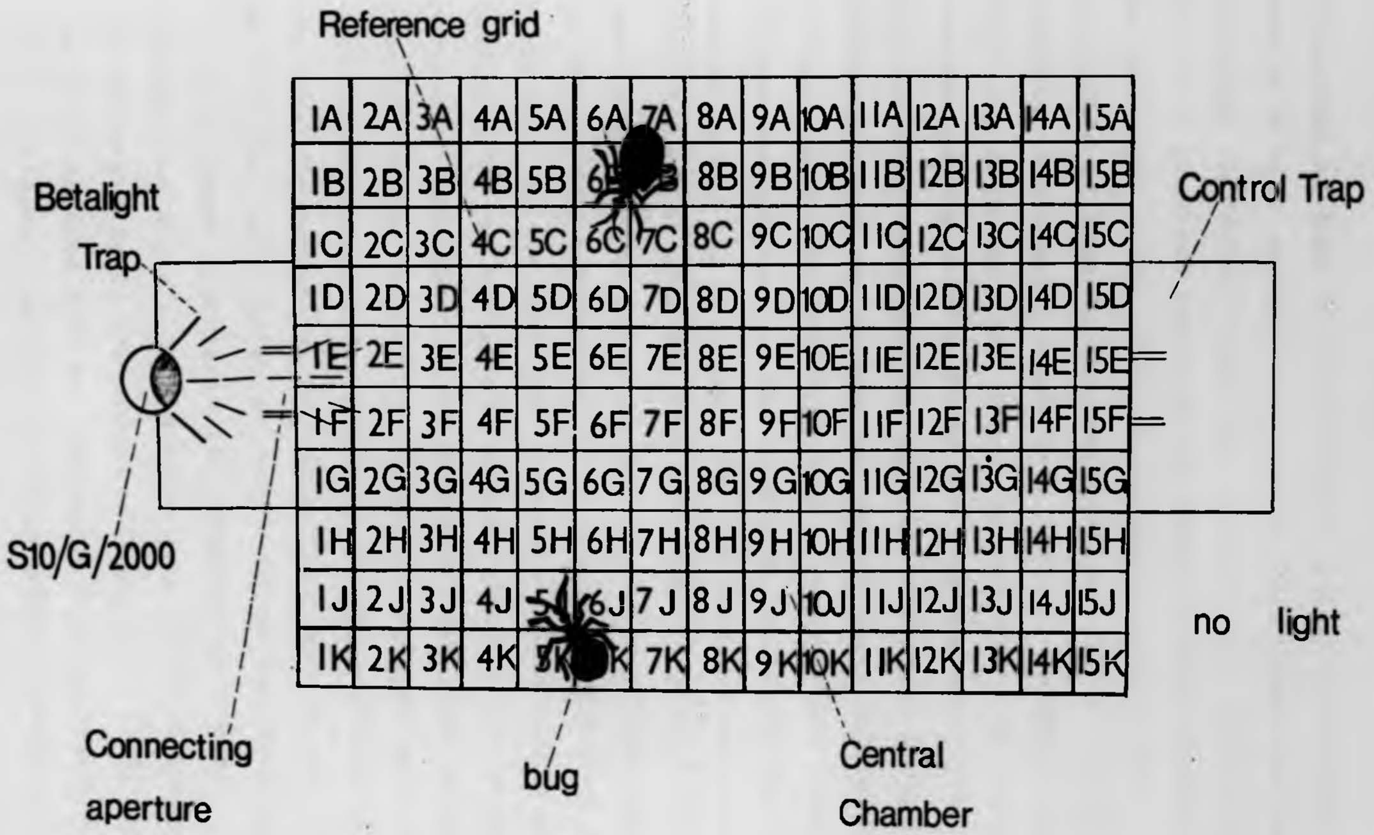


Figure 10. Polythene Choice Chamber-bird's eye view

percentage of bugs in the betalight end of the chamber over a 24 hour period. The results from these experiments, presented in Tables 22 and 23, indicate that during the period of greatest activity and hence maximum response to the betalight source there is an appreciably larger percentage of bugs in the betalight end of the central chamber. Conversely, there is a distinct accumulation of bugs away from the light source in the period of inactivity. Considering the data in Table 23 it is interesting to note that in this experiment the bugs were fed only a short time previously and so were not sufficiently active to actually enter the betalight trap. Nevertheless there is a distinct orientation of the bugs with respect to the light source during the 24 hours. The low level of activity precludes entry into the trap but does not prevent the bugs from aggregating in the betalight end of the chamber. An interesting comparison can be drawn from the data in Table 23. In the Betalight Apparatus with a single green betalight at one end of the chamber there is a distinct diel pattern of orientation with respect to the light source, However, in the Control Apparatus, without any betalight, there is no appreciable difference in the position of the bugs throughout the 24 hours, i.e. there is no light to influence aggregation at either end of the chamber.

In a second experiment the reference grid was divided into three other sections, one of which included all squares A to C, the second all squares D to G, and the third squares H to K. Squares D to G represent the middle horizontal portion of the chamber, an area of the

TABLE 22

Distribution Studies

The distribution of bugs in the central chamber over 24 hours

Time	Number of bugs in Squares 1-8	Number of bugs in Squares 9-15	Total number of bugs in central chamber	Percentage of bugs in Squares 1-8 over 3 hours
0730	1	9	10	6.6
0830	1	9	10	
0930	0	10	10	
1030	1	9	10	16.6
1130	2	8	10	
1230	2	8	10	
1330	3	7	10	30.0
1430	3	7	10	
1530	3	7	10	
1630	5	5	10	41.6
1730	4	4	8	
1830	1	5	6	
1930	2	0	2	80.0
2030	1	1	2	
2130	1	0	1	
2230	1	4	5	52.3
2330	5	2	7	
2430	5	4	9	
0130	3	4	7	36.0
0230	2	6	8	
0330	4	6	10	
0430	1	8	9	10.3
0530	1	9	10	
0630	1	9	10	

TABLE 23

Distribution Studies

The distribution of bugs in the central chamber over 24 hours

	Betalight Apparatus			Control Apparatus		
	Chamber with a single betalight trap - S10/G/2000			Chamber with two lightless traps		
Time	Squares 1-8	Total number of bugs in chamber	% of bugs in 1-8	Squares 1-8	Total no. of bugs in chamber	% of bugs in 1-8
1030	0	10	7.1	9	10	90.0
1130	1	8		9	10	
1230	1	10		9	10	
1330	1	10	20.6	10	10	100.0
1430	1	10		10	10	
1530	4	9		10	10	
1630	2	10	33.3	10	10	100.0
1730	4	10		10	10	
1830	4	10		10	10	
1930	5	10	53.3	9	10	86.6
2030	5	10		8	10	
2130	6	10		9	10	
2230	8	10	64.0	8	10	82.6
2330	4	7		8	10	
2430	4	8		8	9	
0130	2	8	29.1	6	10	73.3
0230	3	8		8	10	
0330	2	8		8	10	
0430	1	9	23.0	10	10	100.0
0530	3	9		10	10	
0630	2	8		9	9	
0730	1	9	3.8	4	8	78.3
0830	0	8		6	7	
0930	0	9		8	8	

TABLE 24

Distribution Studies

The distribution of bugs in the central chamber over 24 hours

Time	Squares D-G	Total number of bugs in central chamber	Percentage of bugs in Squares D - G	Percentage of bugs in Squares D-G over 3 hours
1030	0	10	0.0	3.3
1130	0	10	0.0	
1230	1	10	10.0	
1330	1	10	10.0	3.3
1430	0	10	0.0	
1530	0	10	0.0	
1630	2	10	20.0	13.6
1730	1	7	14.2	
1830	0	5	0.0	
1930	2	4	50.0	58.3
2030	3	5	60.0	
2130	2	3	66.6	
2230	2	3	66.6	47.0
2330	2	6	33.2	
2430	4	8	50.0	
0130	3	8	37.5	28.5
0230	1	4	25.0	
0330	2	9	22.2	
0430	3	9	33.3	13.8
0530	1	10	10.0	
0630	0	10	0.0	
0730	0	10	0.0	0.0
0830	0	10	0.0	
0930	0	10	0.0	

chamber in direct line with the light rays of the betalight source. The aim of this experiment was also to determine whether there was a definite distribution of bugs in the chamber with respect to the light source over a 24 hour period. The results, shown in Table 24, indicate that during the period when the bugs are not active and therefore not responding to betalight there is a distinct accumulation of bugs along the sides of the chamber, particularly in squares A to C or H to K, these being the darker regions of the central chamber. Conversely during the period of activity the percentage of bugs in squares D to G was much greater, indicating a distinct orientation to the betalight source during this period.

The distribution studies carried out in this investigation also indicated another interesting feature of bug behaviour. During the period of inactivity not only was there a tendency for the bugs to accumulate in the darker regions of the chamber but there also appeared to be a distinct 'clustering' phenomenon, i.e. the bugs would tend to aggregate very closely together in the corners of the chamber. At night when the bugs became more active there was a tendency to disperse. This observation was tested by applying a very simple mathematical expression to data relating to bug distribution in the central chamber. The expression is derived from the "nearest neighbour" technique formulated by Clark and Evans (Southwood, 1966). The technique is easy to apply in the case of "discrete easily mapped organisms" and here consisted simply of selecting an individual bug at random and then measuring the distance between it and its nearest neighbour. This procedure was continued until all bugs had been included in the count. The simple expression formulated by Clark and Evans was applied to distribution data relating to a 24 hour observation period which then gave an indication of the density of individuals per unit area.

The expression is:

$$m = \frac{1}{4\bar{r}^2}$$

where m = density per unit area
and r = mean distance between nearest neighbours

Table 25 summarizes the analysis of data on distribution using the Clark and Evans expression. The lower values for m indicate a much greater distance between individuals, in other words a much lower density of individuals per unit area.

The phenomenon of thigmotaxis (also called stereokinesis) has been demonstrated in triatomid bugs. Thigmotaxis has been studied in other insect groups and is reviewed by Wigglesworth (1965). An example of thigmotaxis is easily demonstrated in the earwig <u>Forficula</u>, which when it comes to rest will align its body in such a way that as many of its tactile receptors as possible are brought into contact with any suitable object that is available. Once in this position

TABLE 25

Distribution Studies

Clark and Evans' Expression

Time	r	Total	r^2	$4r^2$	m
1030	1.00	3.90	15.21	60.84	0.0164
1130	1.00				
1230	1.90				
1330	2.00	5.60	31.36	125.44	0.0079
1430	1.80				
1530	1.80				
1630	0.60	8.65	74.82	299.28	0.0034
1730	3.85				
1830	4.20				
1930	3.74	13.54	182.25	729.00	0.0013
2030	2.80				
2130	7.00				
2230	2.87	8.01	64.08	256.32	0.0039
2330	3.58				
2430	1.56				
0130	2.56	11.79	139.24	556.96	0.0017
0230	6.62				
0330	2.61				
0430	2.55	4.55	20.70	82.80	0.0120
0530	1.00				
0630	1.00				
0730	1.00	3.00	9.00	36.00	0.0277
0830	1.00				
0930	1.00				

it is extremely difficult to dislodge the insect and even light, normally eliciting a kinetic effect, ceases to have a stimulatory effect. The inhibitory effect of contact is termed thigmotaxis or stereokinesis. It is therefore interesting to note that during a period of inactivity, and when clustered together, a dim torch light shone on the bugs had little stimulatory effect. Conversely when the bugs are active and have dispersed a light directed on them readily achieves a kinetic effect. These findings may be of particular interest to entomologists involved in the collecting and trapping of triatomid bugs in the field. If attempts are made to search out bugs during the day, perhaps using a strong light to disturb the bugs in their crevice, it is extremely probable that the light will not disturb the bugs sufficiently to cause their emergence. The phenomenon of thigmotaxis may indeed be operative for Pipkin (1968) appears to have met with difficulty in his day time search for bugs. Even the use of pyrethrum dusts proved unrewarding in Pipkin's study, so that ultimately he adopted night collections when the bugs will either emerge voluntarily to obtain a blood meal or alternatively, can be sufficiently disturbed by light shone into their crevices, i.e. when thigmotaxis does not apply. If indeed thigmotaxis does hinder or prevent day time searches for bugs it is possible that the use of light traps might be the only means of collecting sufficiently large numbers of bugs for epidemiological purposes.

The study of distribution within the choice chamber could have been valuably extended by timing the overall approach and fall into the betalight trap in the case of each bug. The bugs, nevertheless, were observed during their response to betalight and the author noticed, in most cases, a fairly slow deliberate approach to the betalight source. This finding is corroborated by Bertram (personal communication) who in a study of adult *Triatoma maculata* gained the impression that the overall rate of approach to the light was slow with very long spells of bugs simply remaining stationary while facing in the direction of the light. It is important to note that the actual speed of movement will change according to ambient temperature. This has been discussed in some detail later in the thesis.

The actual degree of movement in the chamber over the 24 hours will vary according to the bugs' levels of activity. As the bugs become steadily more active so there is a distinct orientation towards the brighter regions of the chamber. During the peak period of activity, and hence phototactic response, there is considerable movement of bugs during each hour. It is important to stress that the level of activity will necessarily depend on the physiological state of the bug and this point has been considered fully later in the thesis. Nevertheless, in Table 25 there is an indication of this movement of bugs judged on values for **r**, the mean distance between nearest neighbours. From 0530 to 1130 the values for r are all the same, namely 1, which means that the bugs have not changed their positions on the grid. Generally, once the bugs have become inactive, according to their photoperiodic entrainment, then each individual bug will remain in virtually the same position during the phase of inactivity. In this case there is very little or no movement during each hour so that a bug might be observed in the same position over a period of seven hours or so. However, from 1130 to 0430 there is some degree of change in the relative position of each individual bug, particularly from 1630 to 0430. There is, albeit the slow deliberate locomotion mentioned previously, a good deal of movement during the hour between observations. The values for r do in fact show a great deal of variability over this 12 hour period. It must be assumed that there has been some degree of movement between observations for the values for r to change so dramatically at each hour.

5. Attraction to other light sources

(i) Introduction

Although this investigation is concerned primarily with the attraction of triatomid bugs to betalight it has also seemed relevant to assess the possibility of using a range of other light sources which could be incorporated into a suitable trapping method. Betalight is a subdued form of illumination which is only readily detectable in darkened situations. The betalight with the greatest brightness used in this investigation had a microlambert value of 2000. Maximum values up to 4000 microlamberts are possible in the betalight range. However, betalight is not a particularly bright form of illumination and can best be described as giving out a subdued glow. A light source with a greater brightness might therefore prove more useful in trapping procedures in the field and the aim of this section of the investigation is to see whether electric light bulbs could be used to attract triatomid bugs and whether they are necessarily more efficient as an attractant. Indeed, a brighter light source than that provided by betalight may be more effective over a greater distance and perhaps more suitable in situations of low level population. Electric light bulbs certainly provide a brighter light source.

(ii) Attraction of adult female Rhodnius prolixus to a blue coloured 25 W lamp

The light source used here was an Osram 'Carnival' 25 W blue coloured lamp (200/250V). In order to determine a diel rhythm of attraction to the light source a polythene choice chamber was used. The blue lamp was

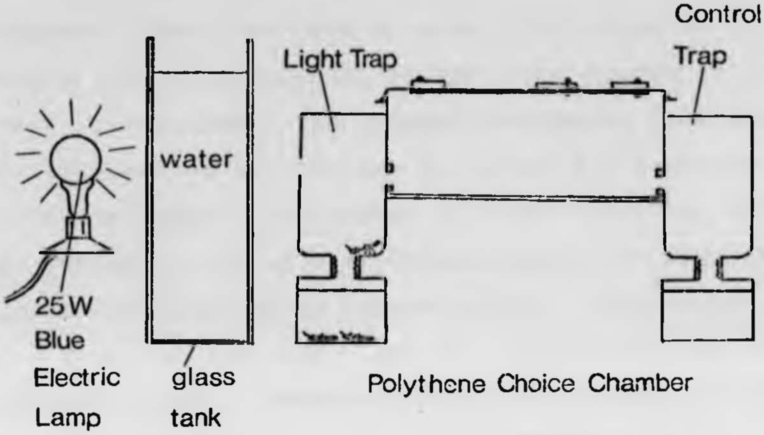


FIGURE 11. APPARATUS USED TO DEMONSTRATE ATTRACTION TO AN "OSRAM" 25 W BLUE COLOURED LAMP

positioned so that its beam was directed through the connecting aperture into the choice chamber of the apparatus. Therefore one trap was lighted and the other constituted a control trap. An experiment was set up to determine a diel rhythm of attraction to the light source by noting the total number of bugs entering the light trap over a 24-hour period. Secondly, it was necessary to determine that this was only a phototactic reaction and not a response to the thermal gradient produced by the lamp. In order to obviate a possible thermal gradient produced by the lamp a large tank of water was placed between the Polythene Choice Chamber and the light source (as shown in Figure 11). In this way a heating effect in the choice chamber could be eliminated.

Attraction to the 'Osram' blue 25 W lamp was indicated and the results are shown over the page in Table 26.

The factor of intensity of the light source was also investigated. This involved no more than noting a comparative ratio of response to the light source at different intensities; the intensities ranged from that of direct illumination and through 1, 2, and 3 thicknesses of white drawing paper. The amount of light resulting after passage through the sheets of drawing paper was measured by using a CdS light meter (Revue S-102). The results shown in Table 26 indicate a greater response at the lower intensity of light. Remembering that the electric lamp has a far greater intensity than that produced by betalight the inverse relationship between illumination intensity and response is to be expected since, as stated in the review of literature on light traps, lights of higher intensity can actually repel. The use of a brighter light source, such as the 25 W blue lamp, is perhaps to be recommended in phototactic responses over a great distance, whereas over shorter distances a dimmer light source could be used to avoid repelling the insects, for example, the siting of betalights on walls near to the bugs' crevices.

The figures presented in Table 26 for the diel rhythm of attraction are those obtained from the experiment where the light intensity was reduced by 47.28%. In this case three sheets of drawing paper were interposed between the choice chamber and the glass tank.

TABLE 26

Diel rhythm of attraction of Rhodnius prolixus to a blue-coloured 25 W lamp

Time	Number of bugs in trap	Percentage attraction	Time	Number of bugs in trap	Percentage attraction
0700-1000	5	8.3	1900-2200	30	50.0
1000-1300	15	25.0	2200-0100	10	16.6
1300-1600	45	75.0	0100-0400	0	0.0
1600-1900	40	66.6	0400-0700	0	0.0

The importance of intensity in the response to a blue-coloured 25 W lamp

Light meter reading in DIN	Number of sheets	Amount of light	Reduction in light intensity	Total number of bugs in trap	Response expressed as a ratio
5.50	0	100.00%	0.00%	21	1.00
5.09	1	92.54%	7.46%	36	1.71
4.10	2	74.54%	25.46%	76	3.61
2.90	3	52.72%	47.28%	145	6.90

6. Determination of the sense organs involved in the response to betalight

(i) Introduction

Betalight is a completely self-powered light source and is said not to produce any heat. Certainly from general handling of betalights it would seem that this is true. However, it could be that betalights produce heat at a level detectable only by bugs and that in the choice chamber experiments the bugs did in fact respond to some kind of thermal gradient. Alternatively, bugs may detect and respond to some element of the radioactivity of the light source, although betalights are said to be entirely safe and free from radioactive leakage, the glass vessel absorbing all of the radiation. The glass vessel is impervious to tritium and absorbs completely any beta-radiation not already absorbed in the phosphor. These two factors therefore, namely heat and beta-radiation, seem very unlikely to constitute part of the response to betalight. Experiments were set up to determine precisely the sense organs involved in the response to betalight.

(ii) Experiments to determine if the antennae are involved in the response to betalight

In a previous investigation using *Triatoma maculata* it was shown that the response to betalight is a purely visual response, for even after removal of the antennae, bugs still show a response to the light source. This suggested that the antennae were not involved in the response and since the thermal senses are located mainly in the antennae (Wigglesworth and Gillett, 1934), it is also suggested that removal of the antennae would impair the response to betalight if indeed some kind of thermal gradient were involved.

Experiments were repeated along the same lines with adult male <u>Triatoma</u> <u>infestans</u>. In one group of twenty bugs the antennae were removed, The antennae were cut off at the base, the scapus being carefully removed. In another group of twenty bugs the antennae were left intact. Each group of bugs was introduced into a polythene choice chamber. The chamber was equipped with a spherical green betalight, S10/G/2000. It is important to remember that the two groups of bugs were of the same nutritional condition. Furthermore, the environmental conditions in the two sets of apparatus were virtually the same, i.e. a temperature of 26.9°C. and a relative humidity of 70 ± 2%.

The total number of bugs entering the betalight traps over a twenty-four hour period was noted. The results are tabulated below:

<u>TABLE 27</u>

	Percentage Attraction to green betalight S10/G/2000	
Time	Bugs with antennae intact	Bugs with antennae removed
0730-1030	5.0	3.0
1030-1330	20.0	15.0
1330-1630	39.0	31.6
1630-1930	28.3	21.6
1930-2230	8.3	5.0
2230-0130	0	0
0130-0430	0	0
0430-0730	0	0

It can be seen from these figures that even bugs in which the antennae have been removed show a response to betalight, albeit not such a good response. Perhaps it is difficult to expect maximum response or indeed 'normal' behaviour in such bugs. Once again it was interesting to note that removal of the antennae did not result in the bugs entering the state of "sleep" or akinesis (Wigglesworth and Gillett, 1934).

Finally response to a betalight source even after removal of the antennae does at least preclude the possibility of photoreceptors on the antennae. In this connection it is interesting to note that Booth (1963) found the antennae of Aphis fabae to be the site of a dermal light sense, although no such mechanism appears to be operative in triatomid bugs.

(iii) To determine the sense organs involved in the response to green betalight, S10/G/2000, in 5th instar nymphs of Rhodnius prolixus

Two Polythene Choice Chambers were used in which the betalight traps were each equipped with a green betalight, S10/G/2000. Fifth instar nymphs of Rhodnius prolixus, fed 29 days before the experiment as 4th instar nymphs, were tested. In order to determine which sense organs are involved in the response to betalight one group of 20 bugs had their compound eyes painted over. The eyes were painted over on the day previous to the experiment. First, the eyes were painted over with Rowney's black acrylic designers' gouache. This had a rubbery texture and adhered well to the surface of the compound eyes. It was noted in a previous investigation that in using black enamel paint for this purpose, as always suggested for entomological experimentation of this kind, it was difficult to obtain a complete and even covering of the eyes. Once the acrylic gouache had dried a coat of Humbrol's black enamel paint was applied to ensure that the bugs were completely blinded. The enamel paint adheres well to the coat of acrylic gouache, the gouache forming a suitable undercoat. At the same time the enamel paint not only ensures another light-proof layer but also provides a protective shell over the surface of the water soluble gouache below. In order to ensure that all facets of the compound eye were equally covered the bugs were examined under a binocular microscope. This group of blinded nymphs was then introduced into a polythene choice chamber.

A second group of bugs which were not blinded was introduced into a second polythene choice chamber. This group constituted a control.

The two groups of bugs were derived from the same colony and were of the same nutritional state. They had been subjected to the same photoperiodic entrainment, i.e. a photophase from 1500-1300 hours, and a scotophase from 1300-1500 hours. The detailed results are presented in Table 29 but a summary is presented below in Table 28.

Table 28

	24-hour catch in betalight trap	24-hour catch in control trap	Totals
Polythene choice chamber 1. Bugs with compound eyes blinded	29	27	56
Chamber 2. Bugs with compound eyes 'normal'	48	11	59
Totals	77	38	115

TABLE 29

Comparing the response of 'blinded' and 'normal' 5th instar nymphs of Rhodnius prolixus to green betalight, S10/G/2000

	Bugs with compound eyes blinded		Bugs with compound eyes normal		
	Polythene choice chamber number 1		Polythene choice chamber number 2		
Time	Betalight trap	Control trap	Betalight trap	Control trap	% Attrn.
0730-1030	2	2	2	0	3.3
1030-1330	12	11	16	2	26.6
1330-1630	9	8	20	5	33.3
1630-1930	6	5	9	3	15.0
1930-2230	0	1	1	1	1.6
2230-0130	0	0	0	0	0
0130-0430	0	0	0	0	0
0430-0730	0	0	0	0	0
Total 24-hour catch	29	27	48	11	

The next step was to ensure that Polythene Choice Chamber number 2 did not in some way favour the entry of a larger number of bugs into the betalight trap. Therefore a repeat of this experiment was set up the following day in which the same 20 blinded bugs were this time placed in Polythene Choice Chamber 2. A summary of the results is presented in Table 30 below.

TABLE 30

	Total number of bugs entering Betalight Trap over a 24-hour period	Total number of bugs entering Control Trap over a 24-hour period
Polythene Choice Chamber number 2		
Bugs with Compound Eyes blinded	16	20

This experiment was repeated several times and always the same pattern emerged. In the case of the blinded bugs there was always a virtually equal number of bugs entering the betalight trap and control trap over a 24-hour observation period. However in the case of a group of bugs with their compound eyes normal there was always a significantly larger number of bugs in the betalight trap suggesting a definite attraction to the betalight source.

The results from these experiments suggest that the sense organs involved in the response to betalight for 5th instar nymphs of Rhodnius prolixus, and indeed all instars of different species, are therefore the compound eyes. The results also indicate that a dermal light sense is not involved and since the nymphs of the Triatominae do not possess ocelli (Usinger, 1944), then the compound eyes can be the only photoreceptors involved.

However, it is important to stress that this must not be taken to suggest a total absence of a dermal light sense in triatomid bugs. There may be such a light sense in bugs and its mechanism and function may be similar to that noted in other groups (Wigglesworth, 1965).

(iv) Location of sense organs involved in response to betalight, S10/G/2000, in adult Rhodnius prolixus

To determine the exact location of the sense organs involved in the response to betalight in adult male *Rhodnius prolixus* the following experiment was set up. Two polythene choice chambers were used in which the betalight traps were each equipped with a green spherical betalight, S10/G/2000. Adult males of *Rhodnius prolixus*, fed 18 days before the experiment, were tested. The bugs had been subjected to the same photoperiodic entrainment, i.e. a photophase from 1500-1300 hours and a scotophase from 1300-1500 hours.

In order to ascertain the exact location of the sense organs involved, one group of twenty bugs had their entire head painted over first with Rowney's black acrylic designer's gouache and then with Humbrol's black enamel paint (this group of bugs being designated as group B). The two coats of paint effectively provided a light-proof layer over both the compound eyes and ocelli, and indeed any other light-sensitive receptors which might be present on the head of the bug. A second group of bugs not subjected to this treatment constituted a control and were designated as group N. The two groups were introduced into two separate Polythene Choice Chambers.

The diel rhythm of attraction to betalight in these two groups was determined over three consecutive days thereby ensuring a number of replicate experiments. These have been designated as experiments A, B, and C. It has already been demonstrated that the two Polythene Choice Chambers used here are identical in experimental performance and in no way offer any bias.

The results obtained from these experiments are tabulated below (Tables 31 and 32). The data indicate that in those bugs whose head had been painted over with two coats of paint (Group B) there is no indication of a diel rhythm of attraction to the betalight source, whereas in those bugs not subjected to treatment (Group N) there is a significant attraction to the light source. The results also preclude the possibility of a general dermal light sense being involved in the orientated response to betalight and indicate that the photoreceptors are at least located at the head end of the bug.

TABLE 31 Experiment demonstrating the location of photoreceptors in adult Rhodnius prolixus

	EXPERIMENT A				EXPERIMENT B				EXPERIMENT C			
	GROUP N		GROUP B		GROUP N		GROUP B		GROUP N		GROUP B	
Time	B.T.	C.T.	B.T.	C.T.	B.T.	C.T.	B.T.	C.T.	B.T.	C.T.	B.T.	C.T.
0700-1000	4	1	0	0	4	2	0	0	6	0	0	1
1000-1300	32	1	3	2	28	2	5	8	33	1	5	2
1300-1600	45	3	3	2	41	1	2	3	38	5	4	4
1600-1900	30	4	2	3	27	2	7	1	27	3	2	4
1900-2200	17	3	1	2	12	5	0	1	19	6	2	5
2200-0100	2	0	0	0	8	1	0	1	4	1	0	0
0100-0400	0	0	0	0	0	0	0	0	0	0	0	0
0400-0700	0	0	0	0	0	0	0	0	0	0	0	0
	130	12	9	9	120	13	14	14	127	16	13	16

ABBREVIATIONS: B.T. = Betalight Trap
C.T. = Control Trap
GROUP N = Bugs not subjected to treatment
GROUP B = Head of bug painted over

TABLE 32

Experiment demonstrating the location of photoreceptors in adult Rhodnius prolixus

Totals of Experiments A, B, and C.

(Mean values in parentheses)

	GROUP N				GROUP B			
Time	Betalight Trap		Control Trap		Betalight Trap		Control Trap	
0700-1000	14	(4.6)	3	(1.0)	0	(0.0)	1	(0.3)
1000-1300	93	(31.0)	4	(1.3)	13	(4.3)	12	(4.0)
1300-1600	124	(41.3)	9	(3.0)	9	(3.0)	9	(3.0)
1600-1900	84	(28.0)	9	(3.0)	11	(3.6)	8	(2.6)
1900-2200	48	(16.0)	14	(4.6)	1	(0.3)	8	(2.6)
2200-0100	14	(4.6)	2	(0.6)	0	(0.0)	1	(0.3)
0100-0400	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
0400-0700	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Totals	377		41		34		39	
Totals of Mean Values		(125.5)		(13.5)		(11.2)		(12.8)

Abbreviations: Group N = bugs not subjected to treatment
Group B = head of bug painted over

The results in Table 32 can be subjected to statistical analysis. For group N the range of the difference of means for p= 0.05 is from 81.84 to 142.12 (the standard deviation of the difference of means σ_d = 14.93 and the difference between means = 112.0; t=2.02). This range does not include zero so the Null Hypothesis has to be rejected. There is therefore a significant difference between the means.

The same method is applied to the data from group B, where the range of the difference of means for p = 0.05 is from - 2.709 to + 5.909 (difference between means = 1.6; σ_d = 2.155 and t = 2.02). Here the range does include zero so there is no significant difference between the means.

(v) The role of the ocelli in the response to betalight

In an experiment on the phototactic responses of adult female *Triatoma maculata* (fed 25 days previously) the relative importance of the ocelli in the response to betalight was assessed. In the previous experiment all the results indicated a localization of photoreceptors at the head end of the bug and a general dermal light sense was precluded. The aim here was to determine whether the two groups of photoreceptors present on the head, namely ocelli and compound eyes, can function separately in the response to betalight.

In one group of 20 bugs (designated as Group 'O') the compound eyes were painted over in the manner described previously. In Group 'O' therefore only the ocelli were operative. In a second group of twenty bugs the ocelli and the compound eyes were left intact. This group was designated as Group 'N'.

The diel rhythm of attraction to green betalight, S10/G/2000 in the two groups of bugs was determined. The results shown in Table 33 indicate that the ocelli by themselves are capable of a phototactic response to betalight. However the ocelli alone are not capable of producing a response to betalight of as great a magnitude as that evident in bugs in which the two groups of photoreceptors are operative. In fact, percentage attraction to betalight never exceeds 25.0% in those bugs with only the ocelli operative.

TABLE 33

To determine if the ocelli are involved in the response to betalight in adult female Triatoma maculata

Percentage attraction to S10/G/2000

	Group 'O' ocelli			Group 'N' compound eyes and ocelli
Time	1	11	111	1
0700-1000	0.0%	0.0%	0.0%	1.6%
1000-1300	6.6%	8.3%	8.3%	48.3%
1300-1600	15.0%	11.6%	15.0%	41.6%
1600-1900	20.0%	25.0%	21.6%	16.6%
1900-2200	6.6%	10.0%	6.6%	3.3%
2200-0100	0.0%	0.0%	0.0%	0.0%
0100-0400	0.0%	0.0%	0.0%	0.0%
0400-0700	0.0%	0.0%	0.0%	0.0%
Total 24-hour catch	29	33	31	67

(vi) The photoreceptors of triatomid bugs

The heads of adult males and females of all species studied here were carefully examined by means of a binocular microscope. This examination indicated the presence of two structurally different groups of eyes in adult triatomids; first, there are the two large compound eyes which have a complex hexagonal arrangement of small facets and second, a pair of much simpler and smaller eyes which are called ocelli. In the 5th, 4th, 3rd, 2nd and 1st instar nymphs of all species there is an obvious absence of ocelli and the compound eyes are noticeably smaller. Whereas in adults the compound eyes are nearly as wide as the interocular space, in nymphs they are approximately 1/6th of the size of the interocular space.

Scanning electron micrographs of the head of a female of Rhodnius prolixus were prepared by Miss M. Backhouse of the Department of Anatomy, University College, London University, in order to gain more information on the external structure of the photoreceptors in triatomids. The scanning electron micrographs are shown in figures 12, 13, 14, 15, 16 and 17. The micrographs are interesting in that they corroborate the experimental findings which indicate only two possible groups of photoreceptors in adult triatomids, namely the compound eyes and the ocelli. Furthermore, in the micrographs of figures 12, 13 and 14 there is no obvious indication of any other type of photoreceptor. Figure 12 is also interesting in that it shows the characteristic arrangement of the light sense organs in the genus Rhodnius, namely that the ocelli are located postero-laterally, behind the compound eyes, on more or less distinct elevations behind a U-shaped suture. (In Triatoma the head does not bear the post-ocular callosity although the ocelli are located postero-laterally on prominent oblique elevations.) Secondly, the head is slightly narrowed at the middle of the anteocular portion.

Figures 15, 16 and 17 show the detailed structure of the compound eyes and in particular the hexagonal arrangement of the facets, an arrangement which is generally assumed in those compound eyes with many ommatidia.

It will of course be interesting to pursue this line of research on the photoreceptors and in particular, the internal anatomy of these structures. For example, it might be informative to know whether the compound eye of the triatomid is of the 'apposition' type or the 'superposition' type. Being nocturnal insects it may be postulated at this stage that it is the latter. This kind of information will be of use in building up a complete picture of phototaxis in these insects.

Figure 12. Scanning Electron Micrograph of Head of female Rhodnius prolixus, showing the characteristic arrangment of the photoreceptors in the genus Rhodnius

Figure 13. Scanning Electron Micrograph of Head of adult Rhodnius prolixus, showing compound eye and ocellus

Figure 14. Scanning Electron Micrograph of Head of adult female of Rhodnius prolixus showing compound eye and ocellus. The tip of the proboscis is also in view

Figure 15. Scanning Electron Micrograph of compound eye showing arrangement of the facets

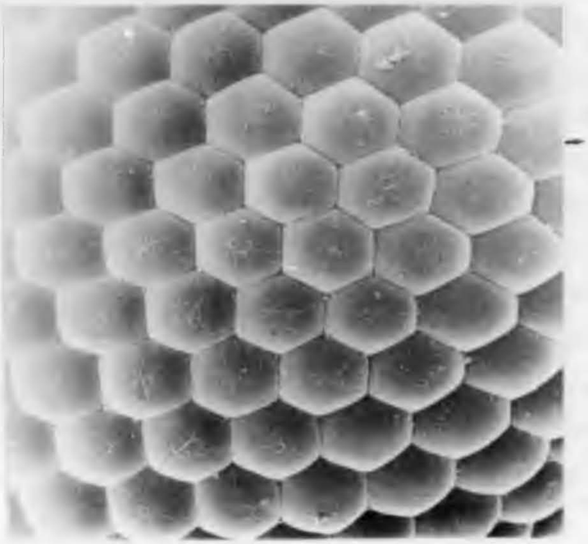


Figure 16. Scanning Electron Micrograph of Head of female Rhodnius prolixus showing compound eye with hexagonal arrangement of the facets

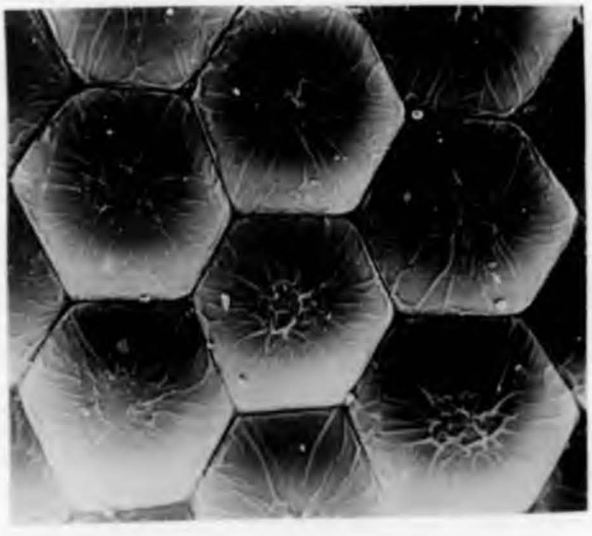


Figure 17. Scanning Electron Micrograph of compound eye showing its characteristic hexagonal arrangement of the facets

(vii) Comparing the response of adult males and adult females of Rhodnius prolixus to green betalight

There are references in the literature to light trap catches where either male specimens or female specimens have predominated in the catch (see review on light trap literature). The experiment described here was therefore set up to compare the response to green betalight, S10/G/2000, in both adult males and adult females of Rhodnius prolixus, ultimately to determine if there is any real difference in the phototactic behaviour of the sexes.

Polythene choice chambers were used and the experimental procedure adopted was that used to determine the diel rhythm of attraction to betalight. The bugs had been fed 14 days before the experiment and had been subjected to the same photoperiodic entrainment.

The results shown in Table 34 are mean values based on three experiments, each of twenty bugs. The results do not indicate any significant difference in the responses of adult males and females. There is no evidence to suggest, therefore, that in the case of triatomids light trap catches in the field will consist predominantly of either sex. Provided the bugs are of the same physiological status, e.g. nutritional history, then there is likely to be no real difference in the response of males and females to any light source. Furthermore, throughout this investigation on the phototactic responses of triatomid bugs, no difference in the phototactic behaviour of males and females was observed.

(vii) Comparing the response of adult males and adult females of *Rhodnius prolixus* to green betalight

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TABLE 34

Comparing the response of adult males and adult females of *Rhodnius prolixus* to green betalight S10/G/2000

	Male *Rhodnius prolixus*	Female *Rhodnius prolixus*
Time	Mean percentage attraction	Mean percentage attraction
0700-1000	2.4	2.4
1000-1300	27.4	29.1
1300-1600	29.1	32.4
1600-1900	29.9	26.6
1900-2200	3.3	2.4
2200-0100	0	0
0100-0400	0	0
0400-0700	0	0

(viii) Comparing the response of adult females and fifth instar nymphs of Rhodnius prolixus to betalight

This investigation was concerned with assessing the response of both fifth instar nymphs and adult females of *Rhodnius prolixus* to a spherical green betalight, S10/G/2000. Experiments to determine the diel rhythm of attraction to betalight were set up to find out if there was any difference in the phototactic responses of nymphs and adults. A group of twenty adult females of *Rhodnius prolixus*, which had emerged 30 days previous to the experiment, was introduced into one polythene choice chamber. A group of twenty 5th instar nymphs, emerged 33 days previous to the experiment, was introduced into a second choice chamber. Each choice chamber was equipped with a spherical green betalight, S10/G/2000.

The nymphs and adults were not given a blood meal after their emergence so that to all intents and purposes they were of the same nutritional condition. It is obviously very difficult to obtain two physiologically identical groups of bugs and there would appear to be no way of ensuring equality of physiological condition other than by the timing of both the blood meal and the emergence. (This point is discussed in more detail later on.) This was therefore the only valuable criterion available to the investigator.

The results obtained from these experiments are shown below in Table 35. The data presented in Table 35 include mean values obtained from three experiments carried out on consecutive days. The mean total percentage response, based on a 9-hour period of activity from 1000 - 1900 hours is 34.07% and 34.25% for adults and 5th instar nymphs respectively. There is no significant difference between these figures which in turn suggests no significant difference in the response of nymphs and adults. Although adult bugs possess two groups of photoreceptors, namely ocelli and compound eyes, there is evidence to suggest that this will not make any substantial comparative difference in the phototactic responses of adults and nymphs, provided that the bugs are of equal nutritional or physiological condition.

This experiment was repeated using adult males and 4th instar nymphs of Triatoma maculata. The nymphs and adults had emerged 32 days previous to the experiment. The following mean total percentage responses were obtained for adults and nymphs respectively, 39.77% and 38.66%. Again there appeared to be no substantial difference in the response of adults and nymphs to a green spherical betalight, S10/G/2000. It therefore seems reasonable to assume that where a trap is used to catch a sample of a wild population of bugs there will be no bias to any particular instar although nutritional condition will in all events be the overriding factor likely to determine the relative numbers of each instar in the catch.

TABLE 35

Comparing the response of adult females and fifth instar nymphs of Rhodnius prolixus to betalight

	Adults			Nymphs		
Time	Total of 3 expts.	Mean	Mean Percentage Attraction	Total of 3 expts	Mean	Mean Percentage Attraction
0700-1000	3	1.0	1.6	2	0.6	1.1
1000-1300	44	14.6	24.4	41	13.6	22.7
1300-1600	77	25.6	42.7	71	23.6	39.4
1600-1900	63	21.0	35.0	73	24.3	40.5
1900-2200	40	13.3	22.2	34	11.3	18.8
2200-0100	0	0	0	0	0	0
0100-0400	0	0	0	0	0	0
0400-0700	0	0	0	0	0	0
Total	227			221		
Mean total percentage response		34.07%			34.25%	

7. Photoperiodic Entrainment

(i) The relationship between the diel rhythm of activity of a triatomid bug and its previous photoperiodic entrainment

In this experiment the diel rhythm of locomotor activity of an adult female of *Rhodnius prolixus* was studied by using an actograph. The bug in this experiment had been subjected to a photoperiodic entrainment with a photophase from 0900-1700 hours.

The actograph, described previously, was connected to a Servoscribe Recorder so that the diel rhythm of activity of the bug could be determined automatically over a period of days. Once the bug had been placed in the actograph it was kept in total darkness over the several days that the experiment was run.

The results are shown in Table 36 on the following page. It will be seen from the data that photoperiodic entrainment would seem to be of paramount importance in determining the onset and duration of activity in adult female *Rhodnius prolixus*, for the period of greatest locomotor activity corresponds to the beginning of the scotophase experienced by the bug in its photoperiodic entrainment. The bugs had been subjected to a scotophase from 1700-0900 hours.

It is also interesting to note at this stage that the onset of locomotor activity begins at approximately the same time during each 24 hour period even though the bug had been kept in a totally dark insectary throughout the run of the experiment.

TABLE 36

Diel rhythm of locomotor activity as determined by actograph

Time	Number of movements	Time	Number of movements	Time	Number of movements
0700-1000	0	1900-2200	15	0700-1000	0
1000-1300	0	2200-0100	7	1000-1300	0
1300-1600	1	0100-0400	0	1300-1600	5
1600-1900	129	0400-0700	0	1600-1900	158
1900-2200	24	0700-1000	0	1900-2200	64
2200-0100	3	1000-1300	0	2200-0100	2
0100-0400	0	1300-1600	7	0100-0400	0
0400-0700	0	1600-1900	147	0400-0700	0
0700-1000	0	1900-2200	10	0700-1000	0
1000-1300	0	2200-0100	2	1000-1300	0
1300-1600	4	0100-0400	0		
1600-1900	140	0400-0700	0		

(ii) The importance of photoperiodic entrainment in determining the onset and duration of the phototactic response in adult female Rhodnius prolixus

Photoperiodic entrainment has been shown to govern the onset and duration of the general activity of the bug. The experiment described here was designed to show how closely the diel rhythm of attraction to green betalight, S10/G/2000, is determined by previous photoperiodic entrainment.

Two photoperiodic entrainment chambers were set up with the following light-dark regimens:

Photoperiodic entrainment chamber 'A'	scotophase 1200-1800 hours photophase 1800-1200 hours
Photoperiodic entrainment chamber 'B'	scotophase 2100-0300 hours photophase 0300-2100 hours

A group of 20 adult female Rhodnius prolixus was introduced into each photoperiodic entrainment chamber and left for 5 days. The temperature and relative humidities within the two chambers were the same, i.e. 27.0 ± 4.0°C. and 65.0 ± 5.0%. After 5 days the two groups of bugs were placed in two separate polythene choice chambers, each equipped with a spherical green betalight, S10/G/2000.

The diel rhythm of attraction to betalight in the two groups, designated as 'A' and 'B', was then determined. The results are shown in Table 37 and Figure 18. There appears to be a significant correlation between the onset and duration of attraction to the betalight source and the previous photoperiodic entrainment experienced by the bugs. The peak of attraction to the light source is attained in the last hour of the period corresponding to the scotophase of the photoperiodic entrainment.

Photoperiodic entrainment played a significant role in these studies. Bugs could be adapted by a minimum of 96 hours exposure to a certain light-dark regimen, i.e. a scotophase from 1300 - 1500 hours, such that although kept throughout a choice chamber experiment in total darkness, the bugs would become active during normal working hours. The bugs' phototactic behaviour could therefore be studied during hours convenient to the investigator. With such an entrainment, the peak of activity would generally occur between 1000 - 1900 hours. The bugs were then returned to the photoperiodic chamber for continuous entrainment between experiments.

Bugs, taken from any other light-dark regimen, could be re-entrained by a minimum of 96 hours exposure to the new reversed photoperiod of 1300 - 1500 hours. This finding is corroborated by Pittendrigh and Bruce (1959) and Beck (1968). Generally the re-entrainment of a behavioural rhythm to a reversed photoperiod cannot be accomplished in one cycle of the rhythm. In the transitional period of from one to several cycles, intermediate responses known as transients become apparent. Although transient responses were not studied in this investigation, nevertheless attainment of the new rhythm in triatomid bugs was achieved within 96 hours, i.e. within several cycles.

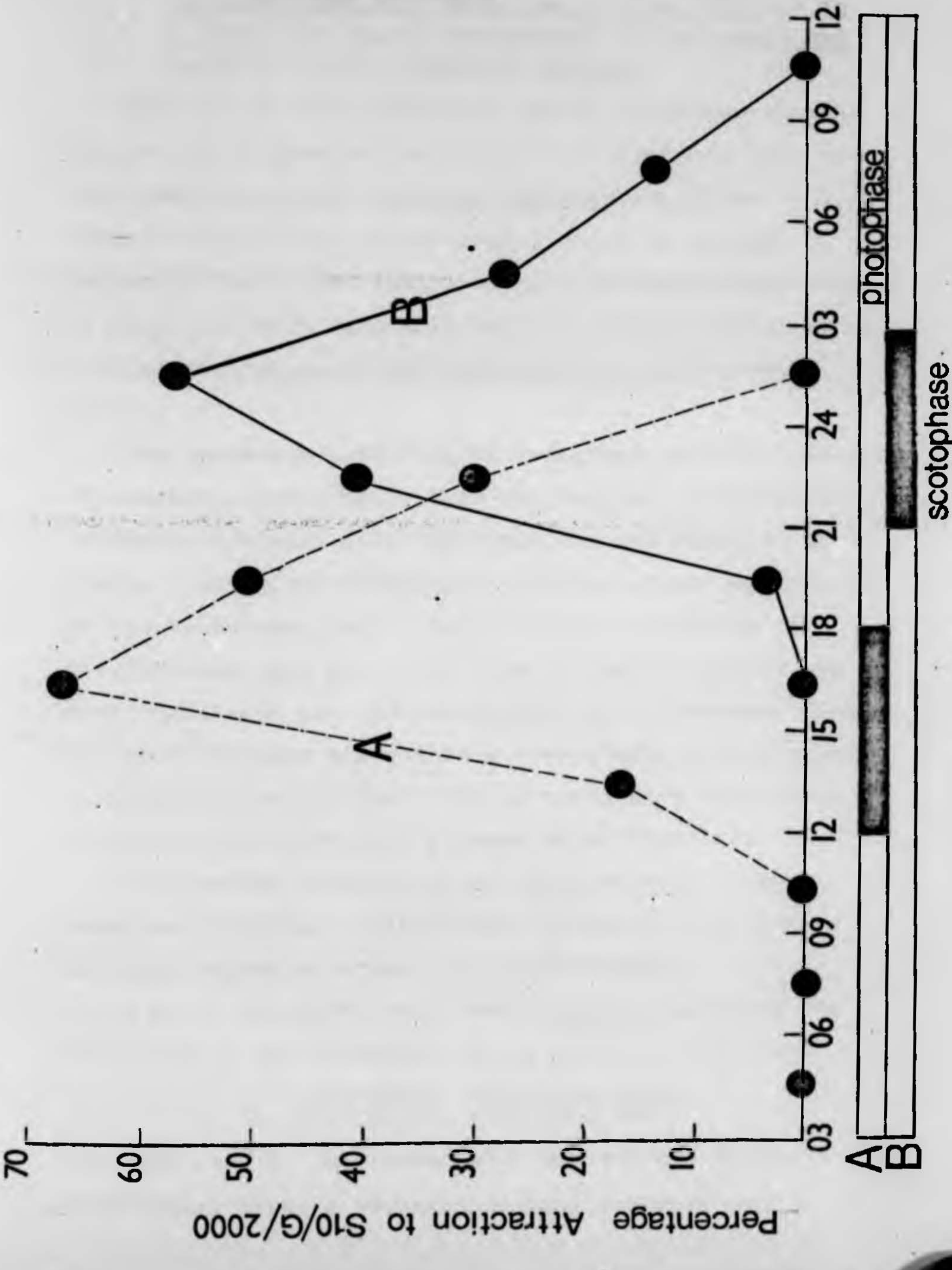
TABLE 37

The relationship between photoperiodic entrainment and the onset and duration of the phototactic response

	Group 'A'	Group 'B'
	Adult female *Rhodnius prolixus* subjected to photoperiodic entrainment with a scotophase from 1200-1800 hours	Adult female *Rhodnius prolixus* subjected to photoperiodic entrainment with a scotophase from 2100-0300 hours
Time	Percentage attraction to S10/G/2000	Percentage attraction to S10/G/2000
0600-0900	0	0
0900-1200	0	0
1200-1500	16.6	0
1500-1800	66.6	0
1800-2100	50.0	3.3
2100-2400	30.0	40.0
2400-0300	0	56.6
0300-0600	0	26.6
0600-0900	0	13.3
0900-1200	0	0

Temperature 26.9°C.
R.H. 70 ± 2.0%

Figure 18. The importance of photoperiodic entrainment in determining the onset and duration of phototactic response in adult female <u>Rhodnius</u> <u>prolixus</u>.



(iii) To determine whether the period during which a triatomid bug will show the maximum response to a betalight source corresponds to the onset and duration of the greatest activity

The aim of this experiment was to determine whether the period of greatest activity of a triatomid bug, in this case adult male Triatoma maculata, could be correlated with the period during which it is most responsive to a betalight source. It seems reasonable to postulate that bugs will only be readily attracted to a betalight source if and when they are sufficiently active.

The apparatus used here to determine the diel pattern of locomotor activity in bugs was set up. Spontaneous locomotor activity of a triatomid bug was measured in a simple rocking box actograph. The actograph was pivoted at the transverse axis slightly above the centre of gravity such that any force (i.e. a bug) at either end would unbalance it. The actograph was so arranged that a pair of mercury dip switches were closed electrically whenever the box tilted. The switches were ultimately connected to a Servoscribe event chart recorder.

A polythene choice chamber equipped with a green spherical betalight, S10/G/2000, was used to determine the diel rhythm of attraction to betalight.

A group of thirty adult male Triatoma maculata was subjected to the following photoperiodic entrainment:

scotophase 1300-1500 hours
photophase 1500-1300 hours

The bugs used in this experiment had emerged 9 days previously, hence a somewhat minimal response to betalight.

One bug was taken from the group of thirty and placed in the actograph. The actograph was maintained in a totally dark insectary in which the temperature and relative humidity were constant over a 24 hour observation period. In this way the onset and duration of activity of the bug could be determined. The number of movements made by the bug in the actograph over a 24 hour period was taken to give an indication of relative activity.

Twenty bugs were placed in a polythene choice chamber and the experimental procedure used to determine the diel rhythm of attraction to betalight was adopted. The chamber was kept continuously in the dark.

Running these two experiments concurrently it was possible to determine whether there was any correlation between the two. The results are shown in Table 38 and figure 19.

Figure 19 shows how the onset and duration of activity as measured by the actograph can be correlated with the diel rhythm of attraction to betalight. The general activity of the bug is governed very precisely by previous photoperiodic entrainment and hence the onset and duration of attraction to betalight closely follows this pattern of activity. The results therefore indicate that bugs are only available to be attracted to betalight if and when they are sufficiently active.

TABLE 38

The relationship between phototactic response and general locomotor activity

Time	Percentage Attraction to S10/G/2000	Number of movements
0900-1200	3.7	44
1200-1500	34.0	79
1500-1800	7.5	3
1800-2100	3.7	0
2100-2400	0.0	0
2400-0300	0.0	0
0300-0600	0.0	0
0600-0900	0.0	0
	Total	126

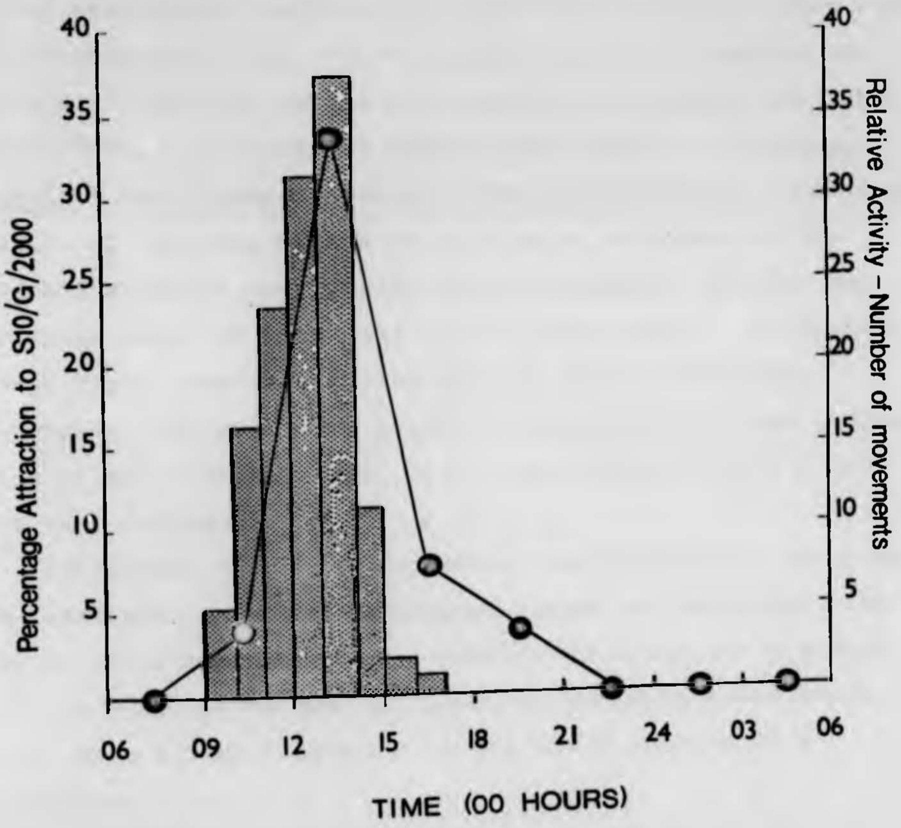


Figure 19. The relationship between phototactic response to green totalight, S10/G/2000, and the diel rhythm of locomotor activity in adult *Triatoma maculata*

notwithstanding the complete absence of a zeitgeber. A free-running rhythm which still shows a distinct periodicity must necessarily depend on some innate time-measuring system and the evidence presented here suggests that triatomid bugs do indeed possess some kind of 'biological clock' which enables a distinct periodicity in phototactic response over a period of several days. An investigation of the biological rhythms of triatomid bugs should prove a rewarding line of research.

TABLE 39

A biological clock in the Triatominae

PHOTOTACTIC RESPONSE TO BETALIGHT

Percentage Attraction to S10/G/2000

Time	DAY 1	DAY 2	DAY 3	DAY 4
0700-1000	6.6%	6.6%	1.6%	5.0%
1000-1300	28.3%	35.0%	26.6%	25.0%
1300-1600	45.0%	50.0%	53.3%	56.6%
1600-1900	45.0%	45.0%	33.3%	43.3%
1900-2200	31.6%	13.3%	28.3%	28.3%
2200-0100	5.0%	0.0%	1.6%	3.3%
0100-0400	0.0%	0.0%	0.0%	0.0%
0400-0700	0.0%	0.0%	0.0%	0.0%

GENERAL LOCOMOTOR ACTIVITY

Total number of movements

Time	DAY 1	DAY 2	DAY 3	DAY 4
0700-1000	1	8	7	8
1000-1300	14	11	23	15
1300-1600	66	67	70	74
1600-1900	7	6	6	4
1900-2200	0	0	0	0
2200-0100	0	0	0	0
0100-0400	0	0	0	0
0400-0700	0	0	0	0

(iv) To demonstrate the presence of a biological clock in triatomid bugs

The importance of photoperiodic entrainment has already been established. In order to determine whether triatomid bugs possess some kind of internal physiological 'clock' which enables them to maintain a distinct rhythmicity in their behavioural patterns in the total absence of time cues or 'zeitgebers', the following experimental procedure was adopted. The diel rhythm of attraction to green betalight, S10/G/2000, in a group of twenty adult males of *Triatoma maculata* was assessed over a period of four days. Over this period of time the bugs were kept in a polythene choice chamber within a continually dark insectary. In this way the bugs were not subjected to any time cues or 'zeitgebers' which might promote a rhythmicity in their behaviour. Naturally, the bugs were exposed continuously to the subdued glow of the betalight but, being continuous, it could not act as a zeitgeber.

Concurrent with this experiment on phototactic response to betalight, a second experiment, using an actograph, was set up to determine the diel pattern of locomotor activity. The aim here was to see if locomotor activity could still manifest a rhythmic pattern in the total absence of a zeitgeber.

The results, presented in Table 39 and Figure 20, indicate that triatomid bugs will still manifest a rhythmic pattern in both their phototactic response to betalight and in their general locomotor activity, notwithstanding the complete absence of a zeitgeber. A free-running rhythm which still shows a distinct periodicity must necessarily depend on some innate time-measuring system and the evidence presented here suggests that triatomid bugs do indeed possess some kind of 'biological clock' which enables a distinct periodicity in phototactic response over a period of several days. An investigation of the biological rhythms of triatomid bugs should prove a rewarding line of research.

TABLE 39

A biological clock in the Triatominae

PHOTOTACTIC RESPONSE TO BETALIGHT

Percentage Attraction to S10/G/2000

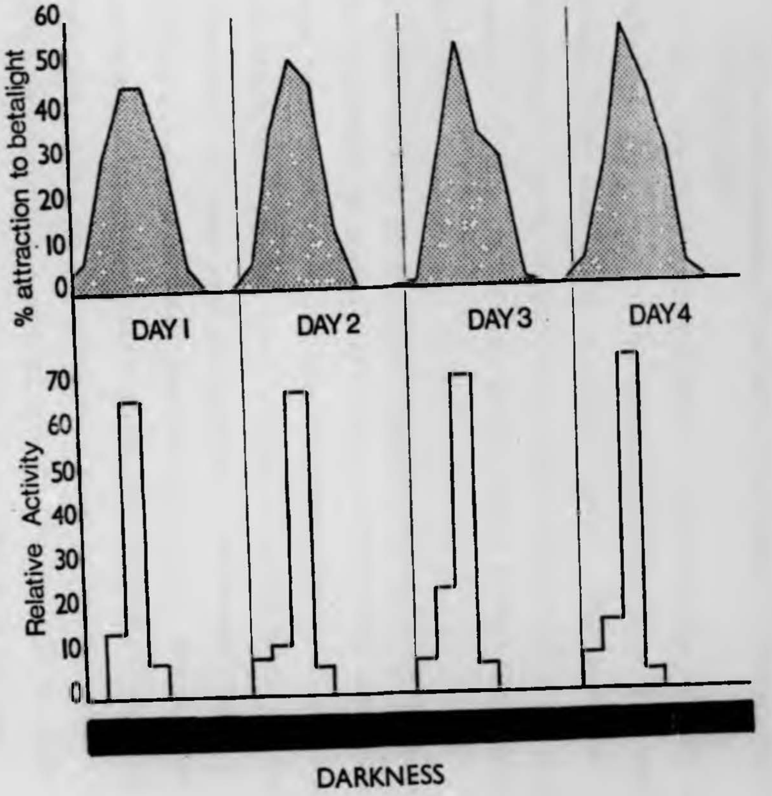
Time	DAY 1	DAY 2	DAY 3	DAY 4
0700-1000	6.6%	6.6%	1.6%	5.0%
1000-1300	28.3%	35.0%	26.6%	25.0%
1300-1600	45.0%	50.0%	53.3%	56.6%
1600-1900	45.0%	45.0%	33.3%	43.3%
1900-2200	31.6%	13.3%	28.3%	28.3%
2200-0100	5.0%	0.0%	1.6%	3.3%
0100-0400	0.0%	0.0%	0.0%	0.0%
0400-0700	0.0%	0.0%	0.0%	0.0%

GENERAL LOCOMOTOR ACTIVITY

Total number of movements

Time	DAY 1	DAY 2	DAY 3	DAY 4
0700-1000	1	8	7	8
1000-1300	14	11	23	15
1300-1600	66	67	70	74
1600-1900	7	6	6	4
1900-2200	0	0	0	0
2200-0100	0	0	0	0
0100-0400	0	0	0	0
0400-0700	0	0	0	0

Figure 20.
A biological clock in Triatominae? A periodicity in behaviour in the absence of a zeitgeber.



8. The physiological status of the bug and its effect on phototactic behaviour

(i) The influence of nutritional history on the response of adult female *Triatoma infestans* to green betalight S10/G/2000

In a previous investigation (Andrewartha, 1972) the important influence of nutritional history on the response to betalight was clearly demonstrated. The earlier experiments were carried out with adult female *T. maculata*. However in this follow-up study adult female *T. infestans* were used.

In order to test the effect of nutritional history on the response to green betalight, S10/G/2000, experiments were performed 1, 8, 14 and 16 days after a blood meal. The magnitude of the diel rhythm of attraction at these intervals of time was calculated. Polythene choice chambers were used here and the betalight traps were equipped with green betalights, S10/G/2000. The bugs had been subjected to a scotophase from 1300-1500 hours in their photoperiodic entrainment. During the period in between experiments the bugs were returned to the Photoperiodic Entrainment Chamber so as to allow continual phase-setting.

It is important to note that although a group of bugs may together be provided with the chance of taking a blood meal on one occasion, there is the possibility that they will not all necessarily feed. Furthermore, it is probable that if the bugs do feed not all of them will feed to the same degree so there is some inevitable biological variation in this procedure. It is therefore difficult to attain a situation in which a group of twenty bugs will all be of the same precise physiological condition.

The data from these experiments are shown below in Table 40 and a graph of the data is shown in figure 21. It can be appreciated from these results that nutritional history has an important influence on the bugs' response to a light source. A few days after a blood meal the bugs are not remarkably active and hence the response to the light source is minimal. However, as the interval after the blood meal lengthens, so there is a progressive increase in activity and thus a gradual increase in the response to the light source.

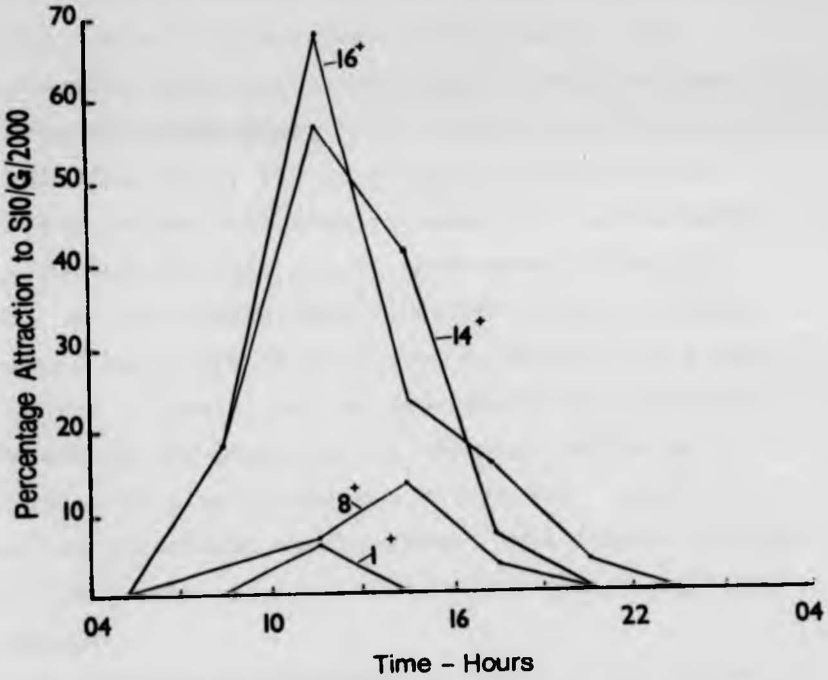
TABLE 40

The influence of nutritional history on the response of adult female *Triatoma infestans* to betalight

PERCENTAGE ATTRACTION TO BETALIGHT

	Days after blood meal			
Time	1	8	14	16
0700-1000	0.0	3.3	18.3	18.3
1000-1300	5.0	6.6	56.6	68.3
1300-1600	0.0	13.3	41.6	23.3
1600-1900	0.0	3.3	6.6	15.0
1900-2200	0.0	0.0	0.0	3.3
2200-0100	0.0	0.0	0.0	0.0
0100-0400	0.0	0.0	0.0	0.0
0400-0700	0.0	0.0	0.0	0.0

Temperature 26.9°C.
Relative Humidity 70.0 ± 3.0%



+ Days after blood meal

Figure 21. The influence of nutritional history on the response of adult female *Triatoma infestans* to green betalight, S10/G/2000.

(ii) The effect of nutritional history on the response of 3rd instar nymphs of Rhodnius prolixus to blue betalight, C135/B/90

As in the preceding experiment the aim here is to determine how the nutritional history of the insect can influence its response to a light source.

Polythene Choice Chambers were used to demonstrate a diel rhythm of attraction to the betalight source, a varying number of days after a blood meal. The experimental procedure is the same as that outlined in the previous experiment.

Third instar nymphs of Rhodnius prolixus were subjected to the following photoperiodic entrainment for a period of eight days: scotophase 1300-1500 hours, and photophase from 1500-1300 hours. A group of twenty bugs were then allowed to take a blood meal and on the following day an experiment was performed to determine the magnitude of the diel rhythm of attraction to blue betalight, C135/B/90. A week later the experiment was repeated, and finally 16 days after that, the response of the same twenty bugs was estimated.

The results are presented in Table 41 and figure 22. The data indicate that nutritional history has an important influence on the bugs' response to betalight, for the more hungry the nymphs are the greater is their response to the betalight source. However, there is one curious difference from the results obtained in the case of adult bugs. Whereas on the day following a blood meal the response to betalight is minimal in adults, in nymphs there is a fairly marked response to the light source. The nymphal instars always appeared to be remarkably active after their blood meal, in marked contrast to the gross inactivity of the adult bugs. This difference in activity levels after a blood meal is a little difficult to explain but it may be assumed at this stage that the blood meal will not have such a pronounced effect on trap catch as it will in the case of adult specimens.

TABLE 41

Nutritional history and its influence on the response to betalight in 3rd instar nymphs of Rhodnius prolixus

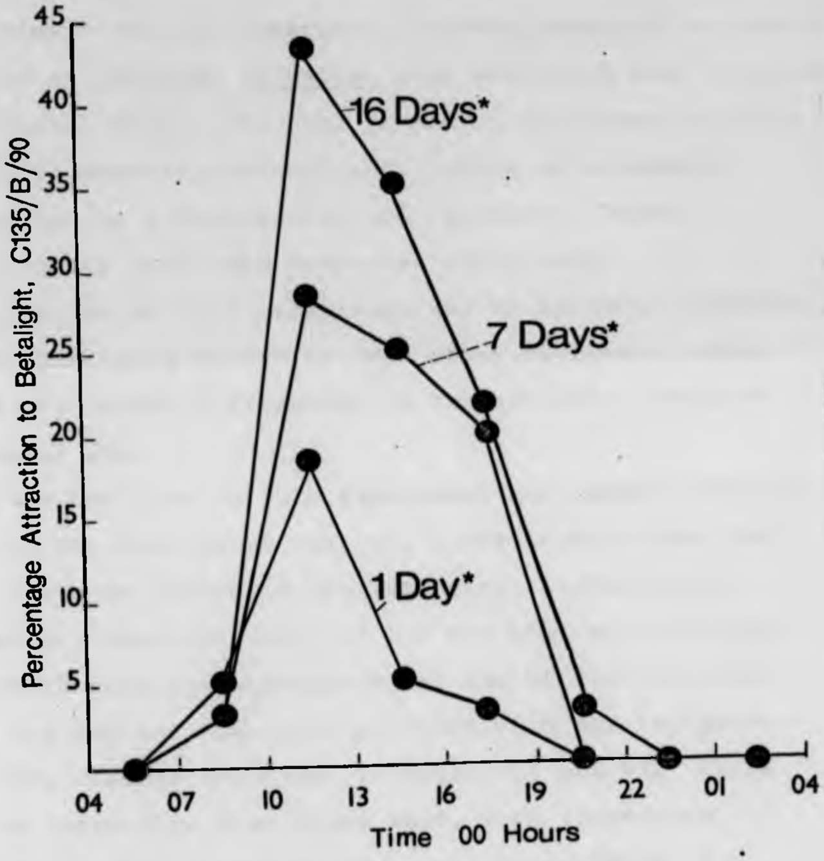
	Percentage Attraction to Blue betalight. C135/B/90		
	Days after blood meal		
Time	1	7	16
0700-1000	5.0%	3.3%	3.3%
1000-1300	18.3%	28.3%	43.3%
1300-1600	5.0%	25.0%	35.0%
1600-1900	3.3%	20.0%	21.6%
1900-2200	0.0%	0.0%	3.3%
2200-0100	0.0%	0.0%	0.0%
0100-0400	0.0%	0.0%	0.0%
0400-0700	0.0%	0.0%	0.0%

adults, in nymphs there is a fairly marked response to the light source. The nymphal instars always appeared to be remarkably active after their blood meal, in marked contrast to the gross inactivity of the adult bugs. This difference in activity levels after a blood meal is a little difficult to explain but it may be assumed at this stage that the blood meal will not have such a pronounced effect on trap catch as it will in the case of adult specimens.

TABLE 41

Nutritional history and its influence on the response to betalight in 3rd instar nymphs of Rhodnius prolixus

	Percentage Attraction to Blue betalight, C135/B/90		
	Days after blood meal		
Time	1	7	16
0700-1000	5.0%	3.3%	3.3%
1000-1300	18.3%	28.3%	43.3%
1300-1600	5.0%	25.0%	35.0%
1600-1900	3.3%	20.0%	21.6%
1900-2200	0.0%	0.0%	3.3%
2200-0100	0.0%	0.0%	0.0%
0100-0400	0.0%	0.0%	0.0%
0400-0700	0.0%	0.0%	0.0%



*Days after blood meal

Figure 22.
The effect of nutritional history on the response of 3rd instar nymphs of Rhodnius prolixus to blue betalight

(iii) Post-emergent activity levels of triatomid bugs

It is postulated that the diel activity levels of a bug are influenced by its metabolic reserves. In this experiment the post-emergent activity levels of an adult female of *Triatoma infestans* were estimated over a period of several days. The diel rhythm of locomotor activity could be measured automatically using an actograph connected to a Servoscribe pen recorder. These instruments have been described previously.

The aim of this experiment was to determine whether, with increasing number of days after emergence, there were any marked differences in the activity levels of an unfed bug.

The bug used in this experiment had emerged 36 days before the experiment was run. Immediately after the bug had been placed in the actograph, lights in the insectary were switched off and the bug was maintained in total darkness throughout the run of the experiment. The bug was not therefore provided with any zeitgeber.

The results are shown in Tables 42 and 43. There is an indication from these that, with increasing number of days after the emergence date, there is a steady increase in the level of activity shown by the triatomid bug. It may be assumed that the progressive increase in activity over several days reflects the progressive decrease in post-emergent metabolic reserves. It may be argued that the increased activity will increase the chances of locating the next potential blood meal.

TABLE 42

Post-emergent activity levels of a triatomid bug

Total number of movements/day

Time	Day 1	Day 2	Day 3	Day 4	Day 5	Total
0100-0400	29	33	49	44	62	217
0400-0700	4	44	30	56	53	187
0700-1000	58	53	32	50	41	234
1000-1300	51	85	90	185	190	601
1300-1600	39	44	32	65	79	259
1600-1900	38	22	18	77	83	238
1900-2200	35	56	49	83	90	313
2200-0100	25	30	59	83	83	280
Totals	279	367	359	643	681	2329

TABLE 43

Post-emergent activity levels of a triatomid bug

	Mean number of movements per day	Time of emergence-number of days before run of experiment
	00.00	1
Day 1	11.62	37
Day 2	15.29	38
Day 3	14.95	39
Day 4	26.79	40
Day 5	28.37	41

(iv) Post-emergent phototactic behaviour in adult Triatoma infestans and Triatoma maculata

In the previous experiment the general locomotor activity of a bug was shown to increase steadily over a period of days following the emergence date.

Preliminary investigations by the author have revealed that newly-emerged adult triatomids manifest only a minimal response to a betalight source whereas those bugs which have emerged some time previously exhibit a response of much greater magnitude. The experiments described here were set up to demonstrate that phototactic behaviour does in fact reflect the post-emergent physiological status of the bug. It has already been proved here in this investigation that phototactic behaviour is a concomitant of locomotor activity, and it seems reasonable to postulate that variations in the latter, as demonstrated in the previous experiment, should influence the levels of phototactic response, e.g. the magnitude of response to betalight a varying number of days following emergence.

Initially, newly emerged adult females of <u>T. infestans</u> and <u>T. maculata</u> were placed in the Photoperiodic Entrainment Chamber. The bugs were entrained with a scotophase from 1300-1500 hours. To assess the varying post-emergent response to green betalight, S10/G/2000, in the two species, experiments to determine the diel rhythm of attraction were carried out using the polythene choice chambers, 11, 18, and 25 days after the emergence date. It is important to note that if bugs were not involved in an experiment they were returned to the Photoperiodic Entrainment Chamber.

The betalight trap in each polythene choice chamber was equipped with a green spherical betalight, S10/G/2000. A total of twenty bugs was introduced into each chamber. The bugs tested in this experiment were not given a blood meal during the post-emergent experimental run.

The temperature in the chamber was 26.9°C. and the relative humidity was 70.0 ± 5.0%

The results of this study are shown in Tables 44, 45 and 46. The results are shown graphically in figures 23 and 24. Newly-emerged bugs exhibit only a minimal phototactic response whereas those bugs which have emerged some time previous, in this case bugs which had emerged 25 days before the experiment, show a much greater response to the betalight source. Clearly, in the absence of a blood meal, the post-emergent physiological status of a bug, i.e. the levels of its metabolic reserves, will influence the degree of locomotor activity and hence phototactic response.

TABLE 44

Post-emergent phototactic responses of triatomid bugs

	(a) Adult female *Triatoma infestans* 11 days after emergence		(b) Adult female *Triatoma maculata* 11 days after emergence	
Time	Betalight Trap	Percentage Attraction	Betalight Trap	Percentage Attraction
0700-1000	0	0.0	0	0.0
1000-1300	6	10.0	4	6.6
1300-1600	5	8.3	3	5.0
1600-1900	3	5.0	0	0.0
1900-2200	0	0.0	0	0.0
2200-0100	0	0.0	0	0.0
0100-0400	0	0.0	0	0.0
0400-0700	0	0.0	0	0.0
Total 24-hour catch in betalight trap	14		7	
Total Catch in Control Trap	0		0	

TABLE 45

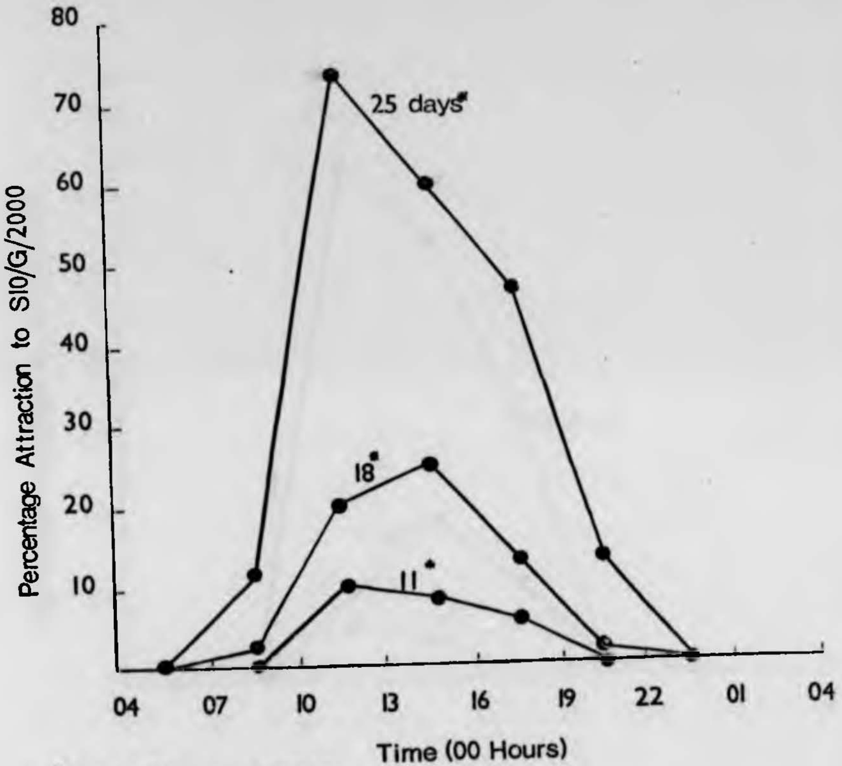
Post-emergent phototactic responses of triatomid bugs

	(a) Adult female *Triatoma infestans* 18 days after emergence		(b) Adult female *Triatoma maculata* 18 days after emergence	
Time	Betalight trap	Percentage Attraction	Betalight trap	Percentage Attraction
0700-1000	1	1.6	0	0.0
1000-1300	12	20.0	6	10.0
1300-1600	15	25.0	12	20.0
1600-1900	8	13.3	7	11.6
1900-2200	1	1.6	1	1.6
2200-0100	0	0.0	0	0.0
0100-0400	0	0.0	0	0.0
0400-0700	0	0.0	0	0.0
Total 24-hour catch in betalight trap	37		26	
Total Catch in Control Trap	3		2	

TABLE 46

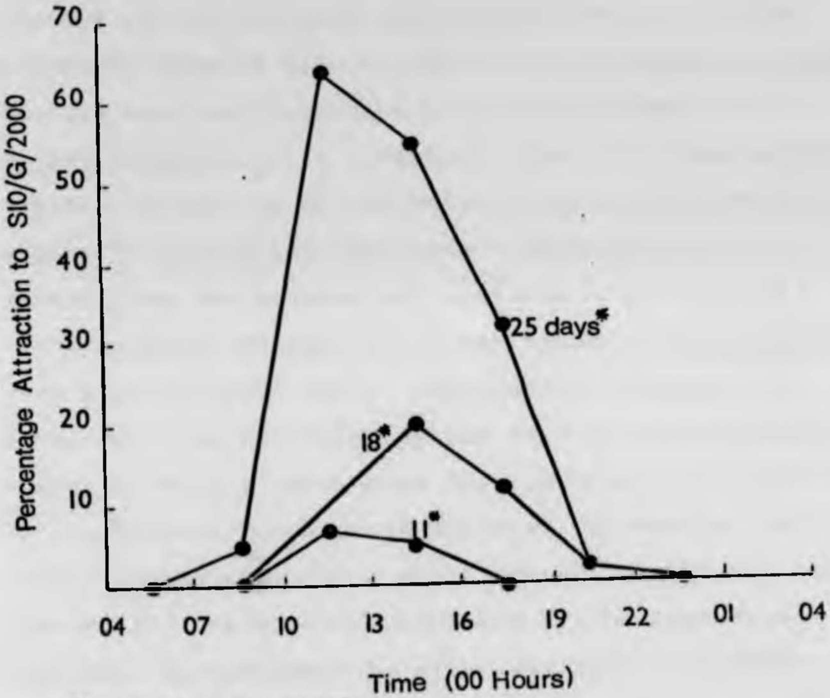
Post-emergent phototactic responses of triatomid bugs

	(a) Adult female *Triatoma infestans* 25 days after emergence		(b) Adult female *Triatoma maculata* 25 days after emergence	
Time	Betalight trap	Percentage Attraction	Betalight trap	Percentage Attraction
0700-1000	7	11.6	3	5.0
1000-1300	44	73.3	38	63.3
1300-1600	36	60.0	33	55.0
1600-1900	28	46.6	19	31.6
1900-2200	8	13.3	1	1.6
2200-0100	0	0.0	0	0.0
0100-0400	0	0.0	0	0.0
0400-0700	0	0.0	0	0.0
Total 24-hour catch in betalight trap	123		94	
Total catch in Control trap	8		6	



*Days after emergence

Figure 23. Post-emergent phototactic responses of adult female *Triatoma infestans*



*Days after emergence

Figure 24. Post-emergent phototactic responses of adult female Triatoma maculata

(v) The phototactic responses of 5th instar nymphs of Triatoma phyllosoma to blue betalight. C135/B/90 during the period following ecdysis

The phototactic behaviour of adult females of *T. infestans* and *T. maculata* following their ecdysis was studied in the previous experiment. The experiment described here is also a study of the behaviour of bugs, in this case newly-emerged 5th instar nymphs of *T. phyllosoma*, over a period of time after the ecdysis or moult. The aim is to determine whether the physiological status following the moult has significance in determining the behavioural patterns of the nymphs.

A group of thirty 4th instar nymphs of *T. phyllosoma* were given a blood meal. Immediately following the blood meal the fully-fed nymphs were photoperiodically entrained with a scotophase from 1300 hours to 1500 hours in the special Entrainment Chamber. As soon as the 4th instar nymphs started to moult through to the 5th instar nymphs, the phototactic responses of the bugs were studied. The response to blue betalight, C135/B/90, was tested 2, 6, 11, 18, 26, 32, and 40 days after the moult. A group of twenty bugs from the thirty entrained bugs, in a polythene choice chamber, was used to assess the response to light on each successive occasion. The bugs were not given a blood meal over the 40 days of the experimental run. Furthermore, the bugs were photoperiodically entrained in the intervals between experiments.

The results are shown in Table 47 and figure 25. There is an indication of a gradual increase in the response to blue betalight over the 40 days following ecdysis. The total number of bugs in the betalight trap (designated as bt in Table 47) at the end of each 24-hour experiment was 9, 12, 22, 25, 33, 40 and 97 at 2, 6, 11, 18, 26, 32 and 40 days respectively after the moult. The diel percentage attraction to betalight at each successive experiment is also shown in Table 47 (designated as pa). Newly-emerged 5th instar nymphs manifest only a minimal response to blue betalight, whereas those which had emerged some 40 days previously can be seen to exhibit a response of much greater magnitude. It is postulated that, in the absence of a blood meal over the 40 days, the bugs had in fact gradually depleted their metabolic reserves with a concomitant increase in activity. The resultant steady increase in activity led to a gradually enhanced response to the betalight source. It may be supposed that the increase in general activity levels of a bug following depletion of its metabolic reserves will considerably increase the chances of it locating the source of its next blood meal. A more mobile bug can obviously cover a much wider area over a given period of time and with that the probability of locating a host must be considerably increased.

Brady (1972) also reports on a steady increase in activity with advancing age and starvation in teneral males of Glossina. This finding clearly corroborates the results obtained in this study.

TABLE 47. Post-emergent phototactic responses of 5th instar nymphs of *Triatoma phyllosoma*

DAYS AFTER EMERGENCE

	2		6		11		18		26		32		40	
	bt	pa	bt	pa	bt	pa	bt	pa	bt	pa	bt	pa	bt	pa
0700-1000	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	3.3	9	15.0
1000-1300	9	15.0	5	8.3	7	11.6	8	13.3	11	18.3	20	33.3	38	63.3
1300-1600	0	0.0	7	11.6	11	18.3	14	23.3	16	26.6	15	25.0	22	36.6
1600-1900	0	0.0	0	0.0	4	6.6	3	5.0	6	10.0	3	5.0	18	30.0
1900-2200	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	10	16.6
2200-0100	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
0100-0400	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
0400-0700	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Totals	9		12		22		25		33		40		97	

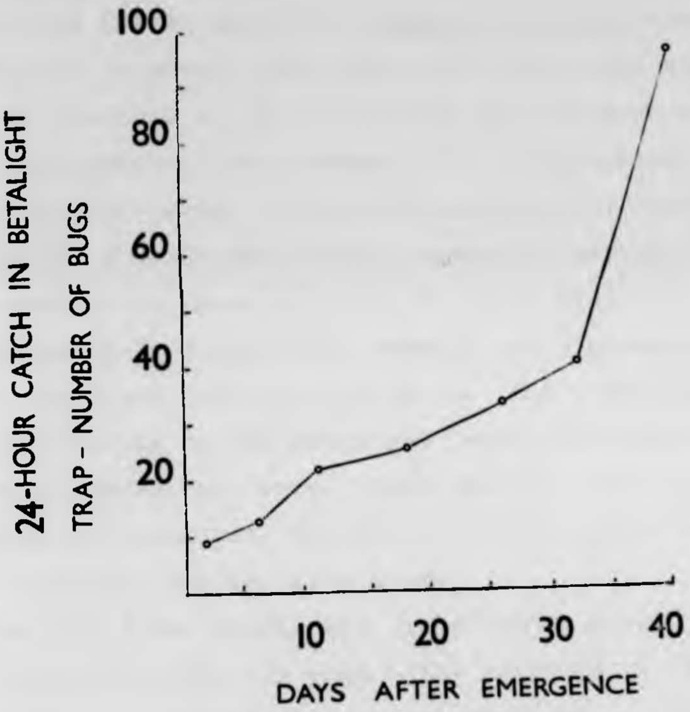


Figure 25. Post-emergent phototactic behaviour of 5th instar nymphs of T. phyllosoma

(vi) The effect of ecdysis on the phototactic behaviour of triatomid bugs

The effect of moulting (ecdysis) on the phototactic response to green betalight, S10/G/2000, was assessed in *Rhodnius prolixus* over a period of nine weeks.

Fourth instar nymphs of *Rhodnius prolixus* were given a blood meal in week 1 (see table 48). The bugs had emerged 4 weeks previous to the blood meal and had been subjected to a photoperiodic entrainment with a scotophase from 1300 hours to 1500 hours. The bugs' phototactic behaviour was studied in a polythene choice chamber at weekly intervals. The results are shown in Table 48 and a graph of the data is presented in Figure 26. Results for 3 experiments, each of 20 bugs, are given in the Table. The total number of bugs responding to the betalight from 1000 hours to 1900 hours was noted each week. From this the total percentage response to betalight, based on a 9 hour period of activity, was calculated for the nine weeks.

In week 3 the nymphs are preparing to moult hence the low value of 8.11%. In week 4 the moult is in progress and the total percentage response to betalight is only 1.27%. In week 5 the newly-emerged 5th instar nymphs yield only a 4.77% response. Gradually from week 5 to week 9 there is an increase in the total percentage response from 4.77% to 62%. The enhanced response to betalight in week 9 may be attributed to the fact that the 5th instar nymphs had been fed some 8 weeks previously as 4th instar nymphs. The bugs were thus in a very active state after this prolonged period of starvation.

The results show that nymphs will moult after only one blood meal although of course the bugs must engorge quite fully with the abdominal integument completely stretched to start the moulting process. The results are also interesting in that they agree with the quantitative data relating to the life cycle of *Rhodnius prolixus* given by Buxton (1930). Buxton found that after feeding 4th instar nymphs of *Rhodnius prolixus* an ecdysis would occur approximately two weeks later. In the experiment presented here nymphs fed in week 1 underwent an ecdysis in week 4 (see Table 48). Generally it was found that an ecdysis would always result 2 to 3 weeks after a blood meal.

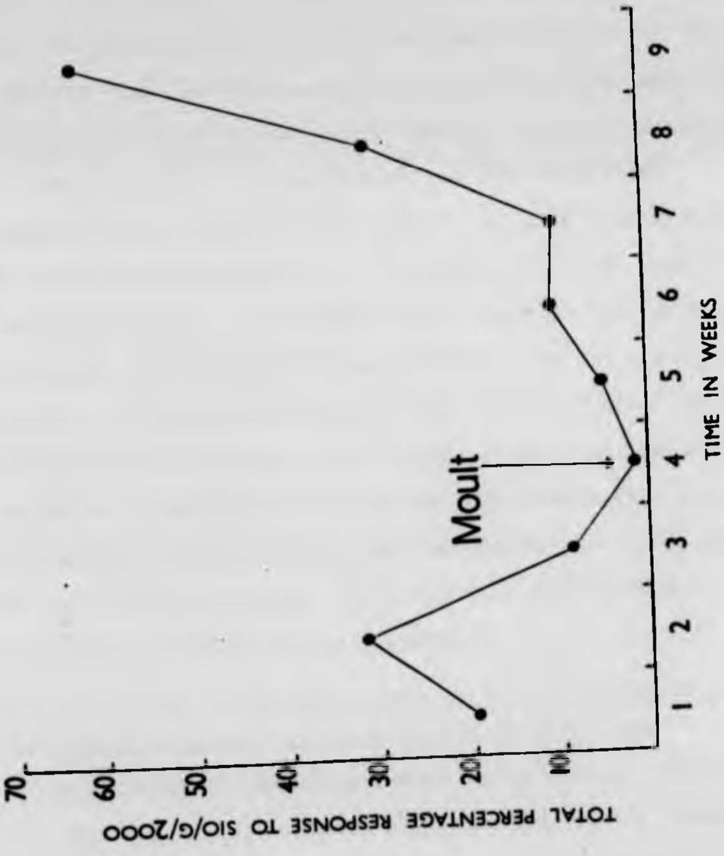
Finally, the decline in the total percentage response in week 3 could probably result from the fact that metabolic reserves might in the week prior to the ecdysis be diverted for the complex process of metamorphosis. That being the case there would not be sufficient reserves for general locomotor activity and hence response to betalight. The physical and 'incapacitating' effect of the actual moult in week 4 will obviously decrease the total percentage response to the light source.

TABLE 48

The effect of ecdysis on the phototactic response of *Rhodnius prolixus* to green betalight

Week	Total number of bugs responding to betalight from 1000 hours to 1900 hours. Expt. 1	Expt. 2	Expt. 3	Mean	Total percentage response
1	37	30	40	35.6	19.77
2	60	52	58	56.6	31.44
3	19	10	15	14.6	8.11
4	4	1	2	2.3	1.27
5	10	8	8	8.6	4.77
6	22	20	15	19.0	10.55
7	20	15	19	18.0	10.00
8	50	58	57	55.0	30.55
9	110	107	118	111.6	62.00

Figure 26. The effect of moulting on the phototactic responses of <u>Rhodnius</u> <u>prolixus</u>



9. The relationship between temperature and response to betalight

(i) General introduction

The influence of temperature on the behaviour of the insect will be discussed in some detail later on. In a laboratory investigation of insect behaviour there are perhaps two ways of studying the influence of temperature. First, it is possible to investigate the complex metabolic processes which ultimately govern the activity of the insect and to see how these are related to the ambient temperature. (It is known that whereas the metabolism of warm-blooded animals is depressed as the external temperature rises, the metabolism of cold-blooded animals such as insects, increases.) Second, one can side-step the more physiological approach and look at the influence of temperature on a behavioural level. In this case the investigator is concerned only with the ultimate effects of temperature and records all aspects of the insect's total behaviour pattern in varying environmental conditions. It was decided to adopt this second approach. The behaviour patterns of triatomid bugs in different temperature conditions were observed and recorded.

(ii) The influence of temperature on the phototactic behaviour of adult female Rhodnius prolixus

The experimental technique was essentially simple. The total number of bugs entering the betalight trap of a polythene choice chamber over a 24 hour observation period was noted. The temperature within the central chamber of the polythene choice chamber could be altered according to the needs of the experimenter by varying the distance between the chamber and an electric heater, in this case a G.E.C. 3 kilowatt floor heater. For example, the nearer the heater was to the Polythene Choice Chamber, the higher the temperature within the central chamber. This simple technique of varying the temperature within the experimental apparatus was used to estimate the effects of temperature on the response to betalight in triatomid bugs.

The Polythene Choice Chamber was equipped with a thermometer and an Edney paper hygrometer so that a close watch could be kept on environmental conditions within the central chamber. The two sets of apparatus were kept in separate insectaries so that two different temperatures could be achieved in the central chambers. In the case of Polythene Choice Chamber 1 the temperature of the central chamber was 15.9°C. and that of Chamber 2, 27.3°C. The relative humidity in the two chambers was 77.0 ± 4.0%.

The betalight traps of the two Polythene Choice Chambers were each equipped with a spherical green betalight, S10/G/2000. With this arrangement any difference in the percentage attraction to betalight could be attributed to the influence of temperature alone, there being no difference in wavelength or brightness in the two light sources.

Adult females of Rhodnius prolixus were used in this preliminary investigation. The bugs were subjected to the following photoperiodic entrainment:

Scotophase 1300-1500 hours
Photophase 1500-1300 hours

The bugs had also been subjected to the same environmental conditions in the Photoperiodic Entrainment Chamber, so that any differences in behaviour could not be attributed to previous environmental conditions. The bugs were fed 28 days before the experiment so that they were quite active and hence readily available to be attracted to a light source.

The experimental procedure was as follows. At 0530 hours bugs were taken from the Photoperiodic Entrainment Chamber and introduced via the perspex tube into each Polythene Choice Chamber. The bugs were not released immediately into the central chamber of the apparatus but were retained in the perspex tube for one hour before the experiment was commenced. It has been variously shown by entomologists that the body temperature of an insect will follow closely that of its surroundings. In the insect at rest, over a moderate temperature range, heat production in metabolism balances heat loss, and the resulting body temperature is the same as that of the surroundings. Furthermore, the body temperatures of insects are known to change very quickly. Hoppers of <u>Locusta</u>, for example, in the shade at 28°C. were found to have a virtually identical body temperature. However on exposure to the sun their body temperature rose to 36°C. in the first minute, finally reaching 42.7°C. in 10 minutes. Once the hoppers had been returned to the shade the body temperature returned to its original 28°C. within 6 minutes (Uvarov, 1948).

The bugs were kept in the perspex tube for one hour so that their body temperatures could attain the same level as that of the experimental ambient temperature.

Unfortunately it was not possible to measure the internal body temperature of the bugs. However, despite the arbitrary period of an hour, the change in body temperature will not require one complete hour. Uvarov's review (1948) indicates a rapid change over a period of a few minutes and it may be supposed that the same is true of triatomid bugs.

At 0630 hours the experiment was started and the two polythene choice chambers were then kept in total darkness over the following 24 hour observation period. The perspex tube was removed and the bugs were thus released into the central chamber. At hourly intervals the number of bugs entering the betalight traps was noted. A note was also made of the temperature and relative humidity in the central chamber.

The results obtained from this preliminary investigation are summarized below in Table 49 and presented in full in Table 50 and figure 27.

TABLE 49

The influence of temperature on the phototactic behaviour of adult female Rhodnius prolixus

	Total Response	Total Percentage Response
Choice Chamber 1 Temperature:- 15.9°C.	6/126	4.76%
Choice Chamber 2 Temperature:- 27.3°C.	48/108	44.44%

It will be remembered that the bugs were subjected to a scotophase from 1300-1500 hours, and so it follows that during this period the bugs should show the

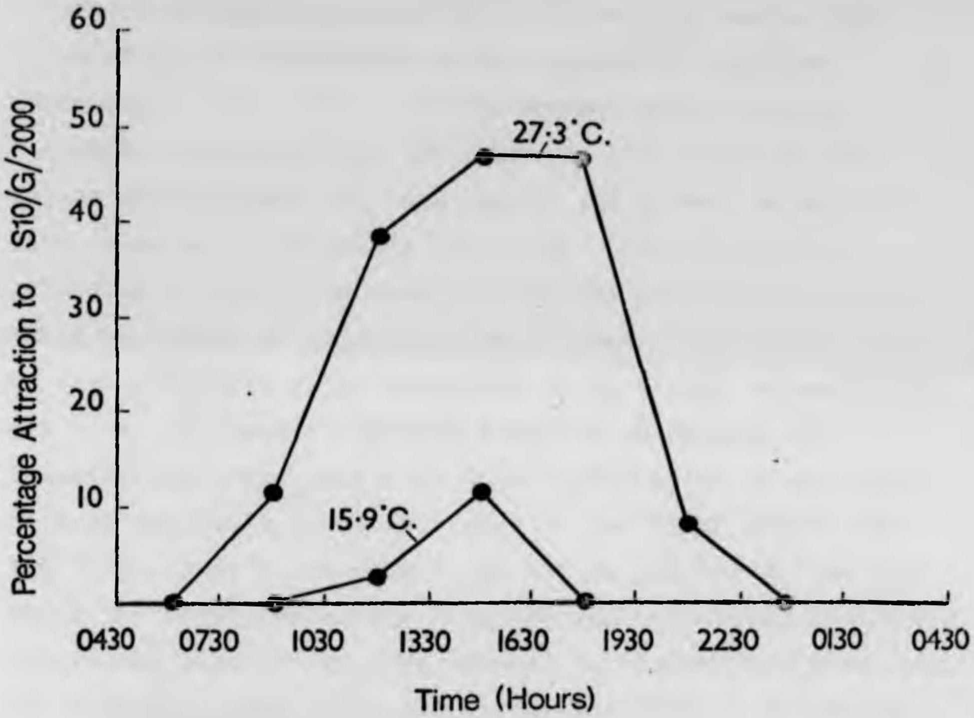


Figure 27.

THE INFLUENCE OF TEMPERATURE ON THE RESPONSE OF ADULT FEMALE <u>RHODNIUS PROLIXUS</u> TO GREEN BETALIGHT, S10/G/2000 greatest activity and hence maximum response to betalight. Considering the data in Table 50 and the graph in figure 27, it will also be seen that maximum response to betalight occurs from approximately 1030 hours to 1930 hours, just a few hours on either side of the period of expected maximum activity. It therefore seemed appropriate to designate, arbitrarily, 1030 hours to 1930 hours as the period during which the bugs should and indeed do manifest their greatest phototactic behaviour. Taking this arbitrary period of 9 hours, the total percentage response, shown in Table 49, could be calculated as follows. The total number of bugs available to be attracted to betalight at each hour was 14 in the case of chamber 1 and 12 in chamber 2. Therefore over the arbitrary 9 hour period the total number of bugs available to be attracted to the light source was 126 in the case of chamber 1 and 108 in chamber 2. On that basis the total percentage response was calculated as 6/126 X 100 in the case of data for chamber 1, remembering that over the 9 hours 6 bugs responded to the betalight. In chamber 2 the total percentage response was 48/108 X 100, since only 48 bugs responded to the light source over the designated 9 hours. From these calculations it was found that the total percentage response was 44.44% at a temperature of 27.3°C. and 4.76% at a temperature of 15.9°C. The results therefore indicate only a fairly minimal response at the lower temperature whereas at the more elevated temperature of 27.3°C. there is a considerably greater response to the same betalight source.

TABLE 50

The influence of temperature on the response of adult female Rhodnius prolixus to green betalight.

S10/G/2000

	Temperature: 15.9°C.		Temperature: 27.3°C.	
Time	Betalight Trap	Percentage Attraction	Betalight Trap	Percentage Attraction
0730-1030	0	0.0	4	11.1
1030-1330	1	2.3	14	38.8
1330-1630	5	11.9	17	47.2
1630-1930	0	0.0	17	47.2
1930-2230	0	0.0	3	8.3
2230-0130	0	0.0	0	0.0
0130-0430	0	0.0	0	0.0
0430-0730	0	0.0	0	0.0
Totals	6		55	
Total in Control trap		2		5

It was interesting to note that in polythene choice chamber 1 with a temperature of 15.9°C. there was a distinct aggregation of bugs at the betalight end of the central chamber. The bugs were not very active and were positioned very closely together. It would appear therefore that even at a low temperature bugs can still manifest a minimal attraction to betalight but because the ambient temperature directly affects the activity of the insect, the bugs will not be sufficiently active to actually enter the betalight trap. A low temperature in the field would result in a low trap catch. It was also noted that the bugs in polythene choice chamber 2, with virtually double the temperature of Chamber 1, were much more active and more easily disturbed by the presence of the investigator.

The conclusion which can be drawn from this preliminary investigation is that as in other insect groups (Cornwell, 1968), low temperatures inhibit the activity of triatomid bugs and high temperatures usually stimulate activity. Since temperature influences the activity of an insect and since the activity of an insect will determine the efficiency of an attractant, insects only responding maximally to the attractant if sufficiently active, it would seem that temperature will be an important environmental factor to consider in any field project which involves extensive trapping of insects.

(iii) The influence of temperature on the response of adult female *Rhodnius prolixus* to blue betalight, C135/B/90

The experimental procedure described above was adopted in this follow-up investigation. Choice chambers 1 and 2 were used once again but in this case the temperature in chamber 1 was 27.3°C. and that in chamber 2 was 15.1°C. By this arrangement it was possible to demonstrate conclusively that neither chamber offered any bias and that both were comparable experimentally.

The results are shown in Table 51 and Table 52. The total percentage response was calculated as described previously on the basis of a maximum activity period from 1030 hours to 1930 hours. A total percentage response of 44.44% is evident at 27.3°C. whereas at 15.1°C. the total percentage response is only 0.92%. This indicates a considerably greater response to blue betalight, C135/B/90, at the more elevated temperature 27.3°C.

TABLE 51

The influence of temperature on the response of adult female Rhodnius prolixus to blue betalight.

C135/B/90

	Choice Chamber 2 Temperature 15.1°C.		Choice Chamber 1 Temperature 27.3°C.	
Time	Betalight Trap	Percentage Attraction	Betalight Trap	Percentage Attraction
0730-1030	0	0	1	2.5
1030-1330	0	0	15	38.4
1330-1630	1	2.7	20	51.3
1630-1930	0	0	17	43.5
1930-2230	0	0	1	2.5
2230-0130	0	0	0	0
0130-0430	0	0	0	0
0430-0730	0	0	0	0
Total 24-hour catch	1		54	
Total number of bugs in Control Trap	0		6	
	r.h.= 77 ± 4%		r.h.= 77 ± 4%	

TABLE 52

	Total response	Total percentage response
Temperature 27.3°C.	52/117	44.44
Temperature 15.1°C.	1/108	0.92

Once again it was noticed that bugs in the chamber maintained at 15.1°C. were not very active and that they remained very close together. The bugs maintained at 15.1°C. did not even respond to the dim light of a torch which was used during the hourly observation periods, whereas those in the chamber maintained at 27.3°C. were agitated at the slightest illumination.

This second experiment was carried out on the day immediately following the experiment described above. Repeating this experiment was useful in that it indicated that even if the bugs had been subjected to 15.9°C. in an experiment on the previous day and shown only a minimal response, that same group of bugs are still able to show a response of greater magnitude at the more elevated temperature of 27.3°C. This would therefore seem to suggest that a bug has the capacity to be active and therefore respond to betalight only if the environmental conditions, and in particular temperature, are suitable. The results from this and the previous experiment certainly indicate that temperature will affect the activity of triatomid bugs and hence govern the attractiveness of a betalight source.

(iv) An investigation of the influence of four different temperatures, 14.5°C., 21.0°C., 27.3°C., and 36.0°C. on the response of adult female Triatoma maculata to green betalight, S10/G/2000

The experimental procedure here is the same as that described for the two experiments described above. Adult female Triatoma maculata, emerged 4 weeks previous to the experiment and not fed as adults, were used in this investigation. The bugs had been subjected to a photoperiodic entrainment in which there was a scotophase from 1300 hours to 1500 hours.

The relative humidity in the central chamber over the experimental run was 77 ± 6%.

The influence of four different temperatures, namely 14.5°C., 21.0°C., 27.3°C. and 36.0°C., on the response of adult female Triatoma maculata to green betalight, S10/G/2000, was tested. The detailed results are presented in Table 54 and the total percentage response, tabulated below, is calculated on the basis of a 9-hour activity period from 1030 - 1930 hours.

TABLE 53

	Total response	Total percentage response
Temperature 14.5°C.	1/180	0.55
Temperature 21.0°C.	49/180	27.22
Temperature 27.3°C.	99/180	55.00
Temperature 36.0°C.	113/180	62.77

The data from Tables 53 and 54 are shown graphically in Figures 29 and 28 respectively. These results indicate the important influence of temperature on the activity of triatomid bugs and hence, their response to green betalight, S10/G/2000, The results also indicate the significant effect of temperature on the phototactic behaviour of a second species of triatomid bug.

The relationship between temperature and total percentage response to betalight, shown in Figure 29, is certainly interesting. There is a steady increase in the total percentage response to betalight with each gradual rise in temperature, to all intents and purposes, a linear relationship. However, between 27.0°C. and 36.0°C., the percentage response is beginning to level off. From these figures it may be possible to assume that maximum activity, and hence maximum response to betalight, will be evident at a temperature of around 36.0°C. In general, it is found that if insects are exposed to a high range of temperature over a period of one hour, they will die between 40°C. - 45°C. (Buxton, 1933). Triatomid bugs exposed to a temperature of approximately 43°C. for one hour are also subject to considerable mortality. After a peak of activity at about 36°C., a gradual falling off in activity is to be expected and, at temperatures above that, possibly a rapid fall in activity and hence a decline in the response to the light source. This decline in activity would result from the harmful effects of high temperatures.

Although the investigator has no data for percentage attraction to betalight between the temperatures of 36.0°C. and 43.0°C., it would seem reasonable to postulate that between these two temperatures there might be a progressive decline in the response to betalight with the imminence of a mortality point at 43°C.

TABLE 54

The influence of four different temperatures, 14.5°C., 21.0°C., 27.3°C., and 36.0°C. on the response of adult female Triatoma maculata to green betalight, S10/G/2000

Time	A Temperature 14.5°C.			B Temperature 21.0°C.		
	B.T.	C.T.	Percentage Attraction	B.T.	C.T.	Percentage Attraction
0730-1030	0	0	0.0	1	1	1.6
1030-1330	0	0	0.0	14	2	23.3
1330-1630	1	0	1.6	19	4	31.6
1630-1930	0	0	0.0	16	0	26.6
1930-2230	0	0	0.0	7	0	11.6
2230-0130	0	0	0.0	0	0	0.0
0130-0430	0	0	0.0	0	0	0.0
0430-0730	0	0	0.0	0	0	0.0
Totals	1	0		57	7	

Time	C Temperature 27.3°C.			D Temperature 36.0°C.		
	B.T.	C.T.	Percentage Attraction	B.T.	C.T.	Percentage Attraction
0730-1030	5	0	8.3	8	3	13.3
1030-1330	29	2	48.3	45	3	75.0
1330-1630	38	7	63.3	36	1	60.0
1630-1930	32	8	53.3	32	0	53.3
1930-2230	10	3	16.6	14	5	23.3
2230-0130	0	0	0.0	1	0	1.6
0130-0430	0	0	0.0	0	0	0.0
0430-0730	0	0	0.0	0	0	0.0
Totals	114	20		136	12	

Abbreviations: B.T. = Betalight Trap
C.T. = Control Trap

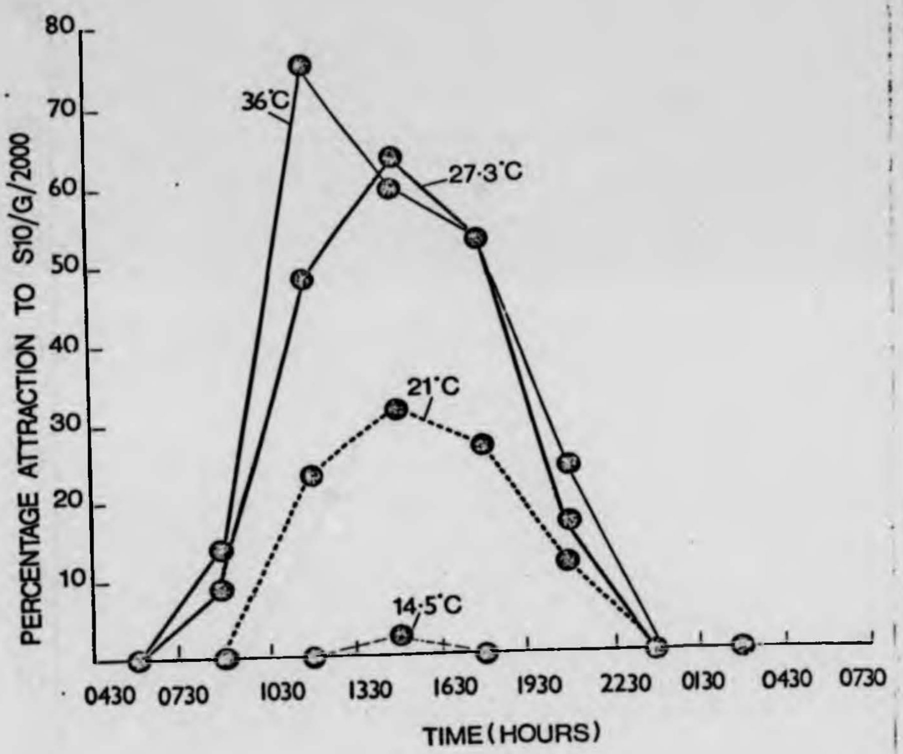


Figure 28. The influence of temperature on the response of 20 entrained adult female Triatoma maculata to green betalight

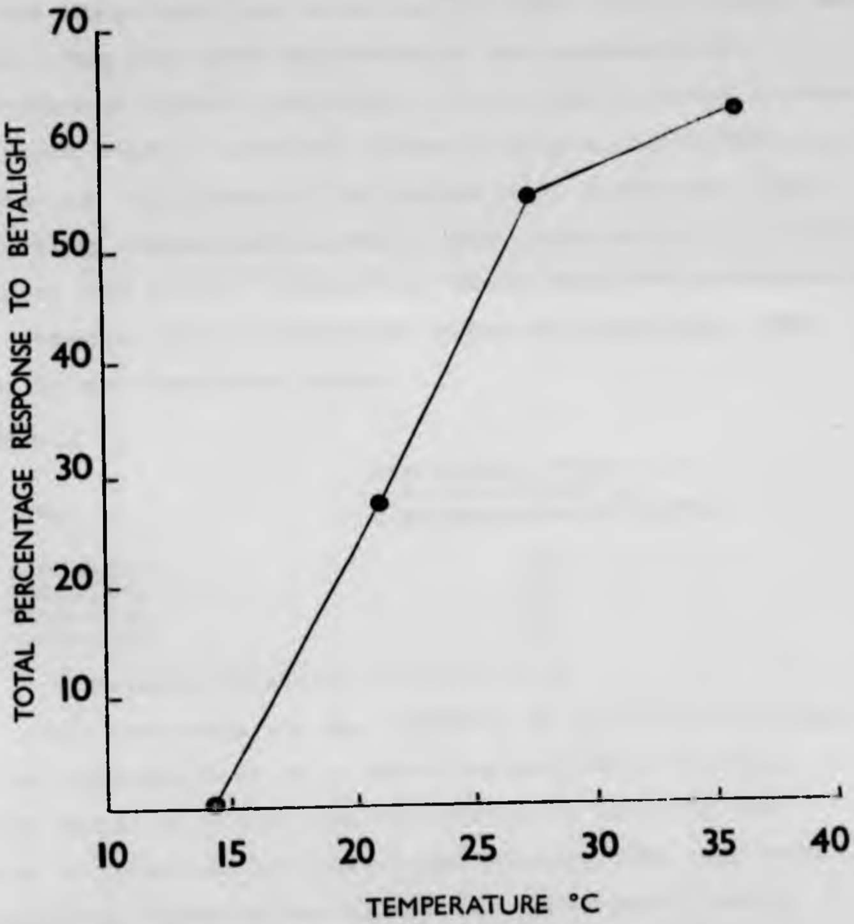


Figure 29. The influence of temperature on the response of adult female T. maculata to green betalight, S10/G/2000.

(v) The effect of a low temperature, 8.5°C., on the behaviour of adult female Rhodnius prolixus

The experimental procedure here was the same as that described in the previous three experiments. Adult female bugs of Rhodnius prolixus which had emerged 14 days previous to the experiment and which had not been fed as adults were used. The bugs were subjected to the photoperiodic entrainment already described. A polythene choice chamber equipped with a spherical green betalight, S10/G/2000, was placed in a light-proof Gallenkamp cool incubator. The resulting temperature in the central chamber of the choice chamber was 8.5°C. A total of twenty bugs was available to be attracted to the betalight source at each hour. The results are tabulated below.

TABLE 55

Time	Percentage Attraction to S10/G/2000 at a temperature of 8.5°C.
0700-1300	0%
1300-1900	0%
1900-0100	0%
0100-0700	0%

Relative humidity = 77.0 ± 1.0%

Bugs show only minimal activity at 15.9°C. so it was to be expected that at a lower temperature of 8.5°C., there would be a complete inhibition of activity and hence no response to a betalight source. The bugs were completely immobile throughout the experiment, gentle prodding with a dissection needle not even stimulating the bugs to move.

At the end of the experiment the bugs were returned to a temperature of 25.0°C. and at this temperature the bugs were found to be alive and completely active. Therefore it would appear from this experiment that exposure to 8.5°C. over a 24 hour period is not fatal for triatomid bugs. This is perhaps not a surprising result for insects in general can survive temperatures many degrees below that which they encounter in nature. Some can also survive prolonged exposure to cold (Buxton, 1933). The desert locust, Schistocerca gregaria, for example, is found to be in a state of cold stupor below 17.0°C., and only begins to resume activity between 17.0°C. and 20.0°C. (Fraenkel and Gunn, 1961). Obviously in the case of adults of Rhodnius prolixus the low temperature of 8.5°C. inhibits activity but is not sufficiently low to result in mortality. It is interesting to note in this connection that at a certain low temperature, varying with the species, insects can become completely immobilized. Mellanby (1939) has described this as the chill-coma temperature. However, the chill-coma temperature, which is the temperature at which the insect is immobilized, must be distinguished from the cold death point, the temperature below which exposure is lethal. A great deal more experimentation is necessary to determine the chill-coma temperature for all triatomid bugs. Certainly for the tsetse fly, Glossina, a temperature of 8.0°C. prevents all movements (Mellanby, 1936), and it would seem that for Rhodnius prolixus, at least, a temperature of 8.5°C. brings about chill-coma (Wigglesworth, 1965). On the basis of these findings it seems reasonable to conclude that a temperature of 8.0°or thereabouts will result in triatomid bugs entering a state of chill-coma.

(vi) The relationship between temperature and locomotor activity in triatomid bugs

The temperature at which maximum locomotor activity may be achieved in triatomid bugs was assessed in the following experiment. The percentage activity of a group of twenty adult *Triatoma infestans* was calculated at twelve different temperatures, ranging from 10°C. to 21.7°C. The different temperatures were achieved by using a Gallenkamp cool incubator. The bugs were kept in the central chamber of a polythene choice chamber which was in turn placed in the incubator. The choice chamber was not equipped with a betalight. The relative humidity in the choice chamber was approximately 70% in each experiment. Bugs were maintained at each respective ambient temperature being tested for one hour before testing their activity. To test activity, each bug in the group was gently stroked on the abdomen with the tip of a camel hair paint brush. A note was made of the total number of bugs which responded to the stimulus at each temperature and from this the percentage activity was calculated. A bug was regarded as being active if it moved only slightly on being subjected to the stimulus. The results shown in Table 56 are based on three experiments. Mean percentage activity ranges from nil at 10°C., to 15% at 16°C., increasing just over two-fold from 36.6% at 18°C. to 81.6% at 19.5°C., and finally achieving 100% at 21.7°C. These results are represented graphically in figure 30. Mellanby (1939) believes that the influence of temperature on the general behaviour of insects is very consistent despite some individual variations and so, from this point of view, the results obtained may be valid for theoretical consideration of those factors likely to influence the trapping of bugs in the field. The fact that a temperature of 21.7°C. gave 100% activity in triatomid bugs agrees with Fraenkel and Gunn's finding (1961) for the desert locust, *Schistocerca gregaria*, which will only become active between 17.0°C. and 20.0°C. It is suggested that locomotor activity in triatomid bugs will only be maximal at or around 21°C. A correlation between locomotor activity and response to betalight has already been drawn and on that basis therefore, excluding the influence of other environmental and physiological factors, it must be supposed that at approximately 21°C., bugs are, at least, sufficiently active to show some kind of response to betalight. However, other experiments in this investigation have indicated that temperatures above 21°C. will considerably enhance the response to betalight. Therefore it is postulated that although activity may be maximal at or around 21°C., above this temperature there is a progressive increase in the degree of locomotor activity and hence a considerably greater phototactic response. A temperature of 21°C. may be accepted for field purposes as a lower limit below which the possibility of trapping bugs steadily declines.

TABLE 56

Percentage activity of adult Triatoma infestans over a temperature range of 10.0°C. - 21.7°C.

Temperature °C.	Number of bugs active out of 20 Expt. 1	Expt. 2	Expt. 3	Mean Percentage activity
10.0	0	0	0	0
11.0	0	0	0	0
12.0	0	0	0	0
13.0	1	0	1	3.3
14.5	2	1	2	8.3
15.0	3	2	4	15.0
16.0	3	4	2	15.0
18.0	7	8	7	36.6
19.0	12	13	10	58.3
19.5	13	15	15	81.6
20.2	16	17	19	86.5
21.7	20	20	20	100.0

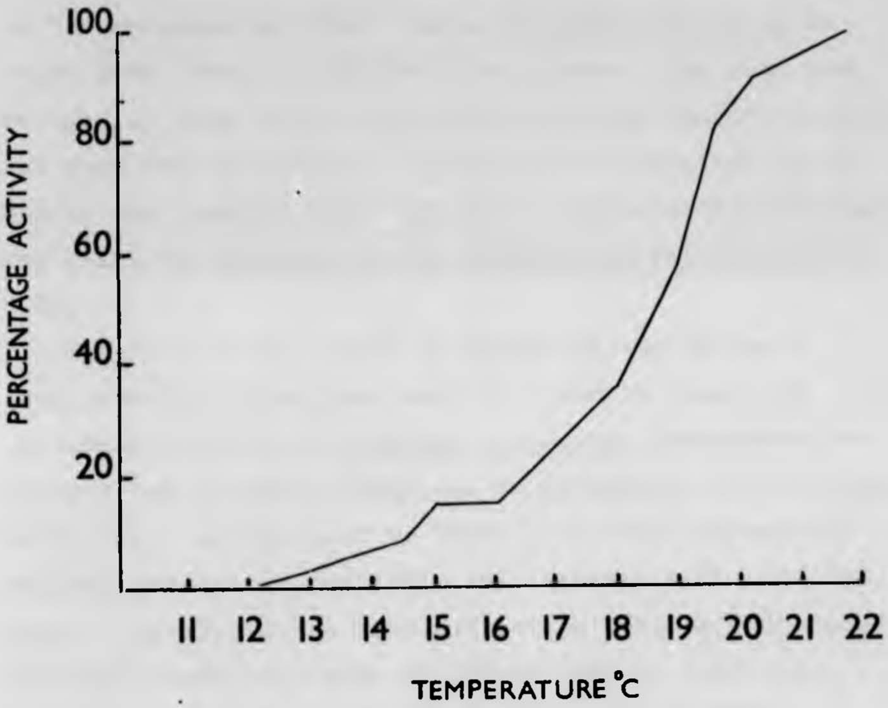


Figure 30. The relationship between temperature and locomotor activity in adult _T. infestans_

(vii) The influence of temperature on the phototactic behaviour of adult female Triatoma infestans

The experimental procedure has been outlined previously. In this experiment the influence of three different temperatures, namely, 7.6°C., 22.0°C. and 27.3°C., on the response of adult female Triatoma infestans to green betalight, S10/G/2000, was tested. The bugs had emerged 17 days before the run of the experiment and had not been fed as adults. The bugs were therefore quite active and readily available to be attracted to betalight. The relative humidity in the central chamber was 77.0 ± 6.0%.

The results are shown in Tables 57 and 58 and a graph obtained from this data is shown in figure 31. In entrained females of Triatoma infestans, post-emergence starved for 17 days, there was no attraction to betalight at 7.6°C., but at 22.0°C. there is a broad plateau of maximum attraction, of 13% - 15%, between 1000 and 1900 hours. At 27.3°C. an 8.3% attraction between 0700 hours and 1000 hours increased to 75% by 1300 to 1600 hours and then declined progressively in the subsequent 3-hourly periods to 58.3%, 15.0%, 1.6% and by 0100 to 0400 hours to a nil response. Clearly, temperature also influences the activity of Triatoma infestans and hence their response to betalight. The low temperature of 7.6°C. brings about a chill-coma in this species, since on subsequently raising the temperature the bugs resume activity. It was stated earlier that a temperature of approximately 8.0°C. may indeed be the chill-coma temperature for triatomid bugs in general. The cold death

TABLE 57

The influence of three different temperatures, 7.6°C., 22.0°C., and 27.3°C. on the response of 20 adult female T. infestans to S10/G/2000

Temperature 7.6°C.

Time	Number of bugs in betalight trap Expt. 1	Expt. 2	Expt. 3	Mean Percentage Attraction
0700-1000	0	0	0	0
1000-1300	0	0	0	0
1300-1600	0	0	0	0
1600-1900	0	0	0	0
1900-2200	0	0	0	0
2200-0100	0	0	0	0
0100-0400	0	0	0	0
0400-0700	0	0	0	0

Temperature 22.0°C.

Time	Expt. 1	Expt. 2	Expt. 3	Mean Percentage Attraction
0700-1000	1	0	2	1.6
1000-1300	8	6	10	13.3
1300-1600	9	10	8	15.0
1600-1900	9	7	11	15.0
1900-2200	2	1	3	3.3
2200-0100	0	0	0	0
0100-0400	0	0	0	0
0400-0700	0	0	0	0

Temperature 27.3°C.

Time	Expt. 1	Expt. 2	Expt. 3	Mean Percentage Attraction
0700-1000	5	2	8	8.3
1000-1300	17	12	22	28.3
1300-1600	45	46	44	75.0
1600-1900	35	33	37	58.3
1900-2200	9	9	9	15.0
2200-0100	1	2	0	1.6
0100-0400	0	0	0	0
0400-0700	0	0	0	0

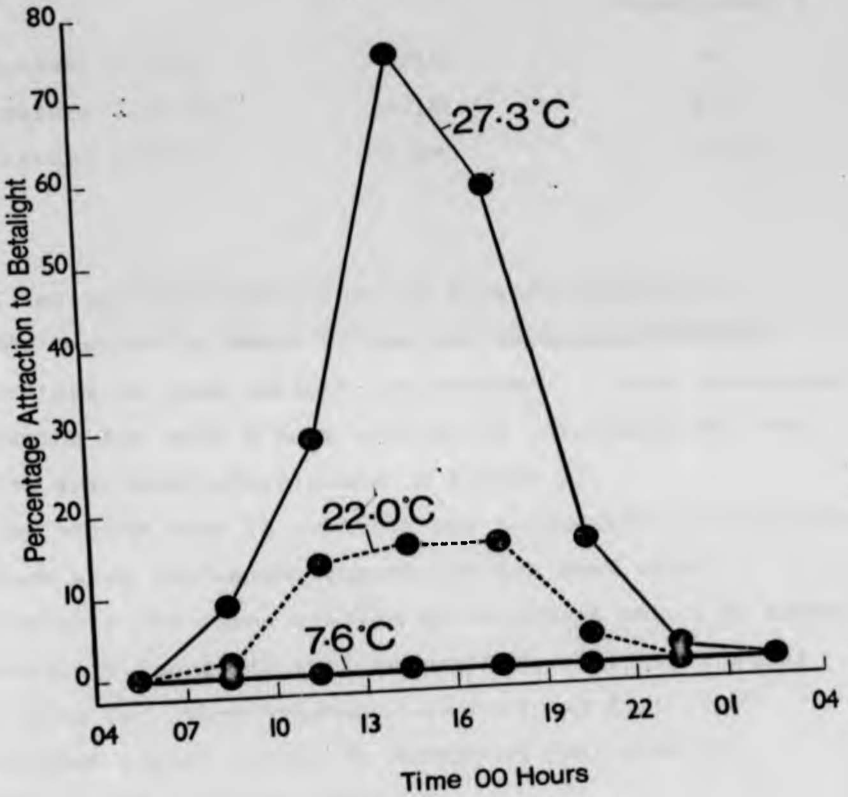


Figure 31.

The relationship between temperature and response to betalight in adult female _Tristoma infestans_

TABLE 58

	Total response for 9 hours	Total percentage response for Experiment 1
Temperature 7.6°C.	0/180	0
Temperature 22.0°C.	26/180	14.44
Temperature 27.3°C.	97/180	53.88

point has not been determined in this investigation.

The results in Table 57 are for three experiments carried out at each ambient temperature. A mean percentage attraction for each 3-hour period was calculated and the results are shown graphically in figure 31.

The steady rise in activity and concomitant phototactic response with increased temperature has been shown conclusively for three species of triatomid bug. It seems reasonable to conclude that temperature will be of equal importance for other triatomid species and that it is an integral factor likely to determine the relative success of any trapping procedure.

(viii) The influence of temperature on the speed of movement in adult female Triatoma maculata

The speed of locomotion or movement of any insect can be estimated by simply recording the time taken for that insect to walk a certain measured distance. In this preliminary experiment the speed of locomotion of an adult female of Triatoma maculata was estimated. The experimental apparatus used for this purpose has been described previously.

The effect of four different temperatures on speed of locomotion was tested, namely, 10.0°C., 14.9°C., 24.1°C., and 27.3°C. The glass chamber of the apparatus used was equipped with a thermometer so that the temperature of the air in the chamber could be read during each experiment. The actual temperature within the chamber could be regulated according to the needs of the experimenter by varying the distance between the chamber and an electric heater, in this case a G.E.C. 3 kilowatt floor heater. In the case of a required temperature of 14.9°C. the experiment was carried out in a second unheated insectary. A low temperature of 10.0°C. was achieved in a Gallenkamp cool incubator. The relative humidity in the chamber was approximately 70% in each experiment.

The bug used in this study had emerged 17 days before the experiment and had not been fed as an adult. The bug had been subjected to a photoperiodic entrainment with a scotophase from 1300 hours to 1500 hours, and correspondingly a photophase from 1500 hours to 1300 hours. The experiment was therefore carried out during the period of maximum activity, between 1300 hours and 1500 hours, corresponding to the scotophase of the entrainment.

The experimental procedure was as follows. At the beginning of the experiment a bug was introduced into the glass cylinder, the aperture between the cylinder and the chamber being closed to prevent the bug entering the chamber immediately. The bug was left for one hour in the cylinder at each ambient temperature being tested, prior to the run of the experiment. As stated previously, one hour was selected as an arbitrary time period and this enabled the body temperature of the insect to be in equilibrium with that of the ambient temperature being tested.

After a period of one hour the bug was released and the time taken for the bug to walk a distance of 22 cm. was noted. Each experiment was carried out in a dark insectary and no attractant was placed in the chamber, i.e. no chemical or light attractant. The aim here was not to determine the time taken to react to and move towards an attractant of any kind, but simply to see how temperature can influence the rate of locomotion.

The bug was marked with a small spot of phosphor powder, type 'C' Batch Sulphide G.E.C. research laboratories, absorbed in paint so that it could be detected in the unilluminated insectary. The limits of the arbitrary distance were also marked with the phosphor powder paint.

Once the bug had moved from the glass cylinder and walked the arbitrary distance of 22 cm., the time taken to cover this distance was noted and the bug was returned to the releasing point. This procedure was repeated many times so that a mean estimate of speed could be obtained. It is important to note that although no attractant, such as betalight, was used in this preliminary experiment, it was therefore necessary on occasion to apply some stimulus to the bug. It has been observed frequently that bugs will not generally move unless provided with a suitable stimulus of some kind or another. Furthermore, the phenomenon of thigmotaxis seems to manifest itself in triatomid bugs. If bugs are not subjected to some kind of stimulus they may therefore, on occasion, come to rest in any suitable corner or crevice of the apparatus. To overcome this problem, on opening the aperture between the cylinder and the chamber, the bug, if stationary, was stroked on the abdomen with the tip of a camel hair paint brush, this mild stimulus encouraging the bug to leave the releasing point and walk the arbitrary distance. Interestingly, McConnell and Richards (1955) in their study of the speed of locomotion in the cockroach found it necessary to stimulate the cerci of the insect in order to induce it to move. In this investigation the stimulus was applied when necessary and so the time taken for the bug to walk the arbitrary distance was thereby estimated.

The results are shown in Table 59 and a graphical representation of the data is shown in figure 32. Rates of movement ranged from immobility at 10.0°C. to a mean of 2.97 cm./sec. at 14.9°C., to 8.42 cm./sec. at 24.1°C., and 10.78 cm./sec. at 27.3°C. The results indicate that the speed of locomotion bears a distinct relationship to temperature and that the actual rate of locomotion is considerably faster at higher temperatures. This finding agrees with that of other investigators, notably Hughes (1965), who have shown that the speed of movement will vary very greatly from one insect to another, but in general is considerably faster at higher temperatures. Mellanby (1939) in his investigation has shown that at each particular temperature above the chill coma point, all 'healthy' insects should move at approximately the same speed and that even though there might be individual variation, in general, behaviour is very consistent.

From these preliminary results it may be possible to postulate that increased response to a betalight source at more elevated temperatures could result from a direct effect on speed of movement. The greater ambulatory activity at higher temperatures enhances the possibility that the bugs might perceive the light source with a resultant increased effectiveness of the light in attracting bugs.

TABLE 59

The influence of temperature on the speed of movement in adult female *Triatoma maculata*

Time taken to walk a distance of 22 cm. at four different temperatures

	10°C.	14.9°C.	24.1°C.	24.1°C.	27.3°C.	27.3°C.
1.	-	9.0 sec.	1.7 sec.	2.0 sec.	1.4 sec.	2.9 sec.
2.	-	4.9 sec.	2.1 sec.	1.9 sec.	2.3 sec.	1.9 sec.
3.	-	9.1 sec.	2.9 sec.	3.0 sec.	2.1 sec.	2.5 sec.
4.	-	5.0 sec.	2.1 sec.	3.2 sec.	3.0 sec.	2.5 sec.
5.	-	8.1 sec.	3.1 sec.	3.0 sec.	1.9 sec.	2.1 sec.
6.	-	8.0 sec.	2.9 sec.	3.0 sec.	1.5 sec.	2.7 sec.
7.	-	8.0 sec.	1.9 sec.	2.4 sec.	1.6 sec.	2.0 sec.
8.	-	8.0 sec.	3.2 sec.	3.0 sec.	2.9 sec.	1.6 sec.
9.	-	7.3 sec.	3.1 sec.	3.1 sec.	1.9 sec.	2.0 sec.
10.	-	6.4 sec.	3.3 sec.	3.1 sec.	2.0 sec.	2.1 sec.
11.	-	7.1 sec.	3.0 sec.	2.9 sec.	2.0 sec.	1.9 sec.
12.	-	8.0 sec.	2.1 sec.	2.1 sec.	1.9 sec.	1.9 sec.
Mean	-	7.4 sec.	2.6 sec.	2.7 sec.	2.0 sec.	2.1 sec.
Mean Speed cm./ sec.	0	2.973	8.429		10.78	

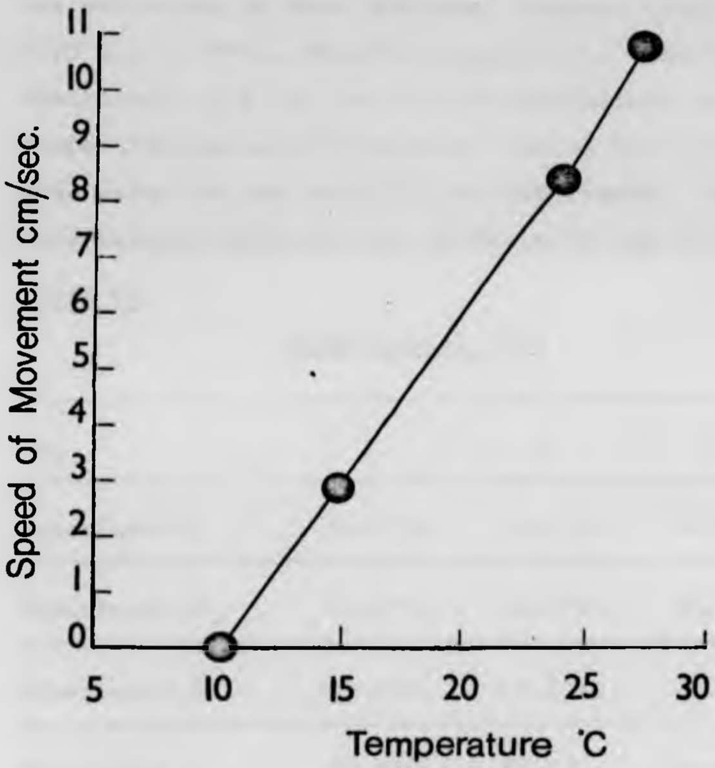


Figure 32. The influence of temperature on the speed of movement in Triatoma maculata

ix. The relationship between temperature and speed of movement in adult female *Triatoma infestans*

The experimental procedure adopted here is the same as that described in the previous experiment. The speed of movement of three adult females of *Triatoma infestans* was estimated at four different temperatures, namely, 10.0°C., 14.9°C., 24.1°C., and 27.3°C. The bugs, designated as A, B, and C, were maintained at each respective ambient temperature tested for a total of one hour prior to the start of the experiment. The experimental plan set out in Table 60 was followed.

TABLE 60

Experimental Plan

Bug	A	B	C
Experiment 1	24.1°C.	10.0°C.	14.9°C.
Experiment 2	10.0°C.	14.9°C.	27.3°C.
Experiment 3	14.9°C.	27.3°C.	24.1°C.
Experiment 4	27.3°C.	24.1°C.	10.0°C.

It will be seen that four experiments were set up to assess the influence of temperature on the speed of movement in each individual bug. Each experiment in turn consisted of 20 observations to note the speed of movement in bugs A, B, and C.

The entire experiment was started at 1200 hours which corresponded to the beginning of the period of activity as governed by photoperiodic entrainment. The bugs had been subjected to a scotophase from 1300 hours to 1500 hours.

The results are shown in Tables 61, 62 and 63 and a graphical representation of the data is shown in figure 33. The data presented in Tables 61, 62 and 63 are records of the time taken for each bug to walk a distance of 22 cm. at four different temperatures. Rates of movement (based on data for the three bugs) ranged from immobility at 10°C., to a mean of 3.0 cm./sec. at 14.9°C., of 5.7 cm./sec. at 24.1°C. and 8.5 cm./sec. at 27.3°C. Once again temperature can be seen to exert a distinct influence on speed of movement. The increased speed of movement at more elevated temperatures may therefore account for the greater magnitude of response to betalight which is evident in situations with a high temperature. A full consideration of the way in which temperature may indeed bring about these fluctuations in the response to betalight is presented in the Discussion.

TABLE 61

The influence of temperature on the speed of movement in adult female Triatoma infestans

BUG "A"

Experiment Number	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Temperature °C.	24.1	10.0	14.9	27.3
1.	3.5 sec.	-	8.1 sec.	1.5 sec.
2.	3.1 sec.	-	8.0 sec.	1.0 sec.
3.	2.1 sec.	-	15.5 sec.	1.3 sec.
4.	4.9 sec.	-	12.9 sec.	2.0 sec.
5.	5.0 sec.	-	6.4 sec.	2.0 sec.
6.	5.0 sec.	-	7.0 sec.	1.1 sec.
7.	3.9 sec.	-	9.2 sec.	1.5 sec.
8.	4.9 sec.	-	8.0 sec.	1.5 sec.
9.	3.9 sec.	-	9.9 sec.	2.0 sec.
10.	3.0 sec.	-	7.9 sec.	1.0 sec.
11.	4.0 sec.	-	7.9 sec.	3.0 sec.
12.	4.6 sec.	-	6.2 sec.	2.7 sec.
13.	4.1 sec.	-	9.5 sec.	3.9 sec.
14.	2.5 sec.	-	15.0 sec.	2.7 sec.
15.	2.5 sec.	-	12.5 sec.	2.3 sec.
16.	4.5 sec.	-	11.6 sec.	1.0 sec.
17.	4.9 sec.	-	12.5 sec.	2.0 sec.
18.	4.2 sec.	-	9.2 sec.	2.5 sec.
19.	3.0 sec.	-	8.7 sec.	2.0 sec.
20.	3.0 sec.	-	12.7 sec.	3.5 sec.
Totals	76.6 sec.	-	198.7 sec.	40.5 sec.
Mean	3.83 sec.	-	9.93 sec.	2.02 sec.
Mean Speed in cm/sec	5.744	0.000	2.216	10.89

TABLE 62

The influence of temperature on the speed of movement in adult female Triatoma infestans

BUG "B"

Experiment Number	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Temperature °C.	10.0	14.9	27.3	24.1
1.	-	9.0 sec.	4.1 sec.	2.9 sec.
2.	-	9.0 sec.	2.0 sec.	4.0 sec.
3.	-	10.0 sec.	3.0 sec.	3.7 sec.
4.	-	7.2 sec.	3.9 sec.	4.0 sec.
5.	-	9.0 sec.	2.5 sec.	3.1 sec.
6.	-	6.0 sec.	4.0 sec.	3.9 sec.
7.	-	8.1 sec.	2.0 sec.	4.0 sec.
8.	-	7.8 sec.	3.0 sec.	4.1 sec.
9.	-	8.0 sec.	3.5 sec.	4.1 sec.
10.	-	9.1 sec.	3.7 sec.	3.1 sec.
11.	-	10.0 sec.	3.0 sec.	3.1 sec.
12.	-	7.5 sec.	2.8 sec.	5.0 sec.
13.	-	9.1 sec.	4.0 sec.	3.5 sec.
14.	-	8.0 sec.	3.5 sec.	5.0 sec.
15.	-	13.5 sec.	3.1 sec.	3.0 sec.
16.	-	12.0 sec.	3.1 sec.	4.1 sec.
17.	-	9.9 sec.	2.5 sec.	3.2 sec.
18.	-	8.0 sec.	3.0 sec.	2.5 sec.
19.	-	9.1 sec.	3.1 sec.	3.5 sec.
20.	-	10.0 sec.	3.1 sec.	3.5 sec.
Totals	-	180.3 sec.	62.9 sec.	73.3 sec.
Mean		9.01 sec.	3.14 sec.	3.66 sec.
Mean Speed cm/sec.	0.000	2.442	7.006	6.010

TABLE 63

The influence of temperature on the speed of movement in adult female Triatoma infestans

BUG "C"

Experiment Number	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Temperature °C.	14.9	27.3	24.1	10.0
1.	2.9 sec.	2.7 sec.	4.1 sec.	-
2.	15.0 sec.	2.0 sec.	5.0 sec.	-
3.	6.0 sec.	2.0 sec.	6.0 sec.	-
4.	3.0 sec.	3.6 sec.	5.0 sec.	-
5.	3.2 sec.	2.9 sec.	4.0 sec.	-
6.	3.0 sec.	3.9 sec.	3.5 sec.	-
7.	3.9 sec.	2.5 sec.	4.0 sec.	-
8.	4.9 sec.	3.2 sec.	3.2 sec.	-
9.	4.8 sec.	3.0 sec.	4.8 sec.	-
10.	3.0 sec.	4.1 sec.	3.9 sec.	-
11.	4.6 sec.	2.0 sec.	3.0 sec.	-
12.	3.9 sec.	2.3 sec.	3.5 sec.	-
13.	4.9 sec.	3.7 sec.	2.5 sec.	-
14.	5.0 sec.	3.0 sec.	3.1 sec.	-
15.	6.0 sec.	2.2 sec.	4.5 sec.	-
16.	4.3 sec.	2.1 sec.	4.0 sec.	-
17.	4.0 sec.	3.4 sec.	5.0 sec.	-
18.	9.0 sec.	3.0 sec.	4.7 sec.	-
19.	4.4 sec.	2.9 sec.	4.0 sec.	-
20.	5.5 sec.	3.0 sec.	4.0 sec.	-
Totals	101.3 sec.	57.5 sec.	81.8 sec.	-
Mean	5.06 sec.	2.87 sec.	4.09 sec.	
Mean Speed cm/sec.	4.347	7.665	5.379	0.000

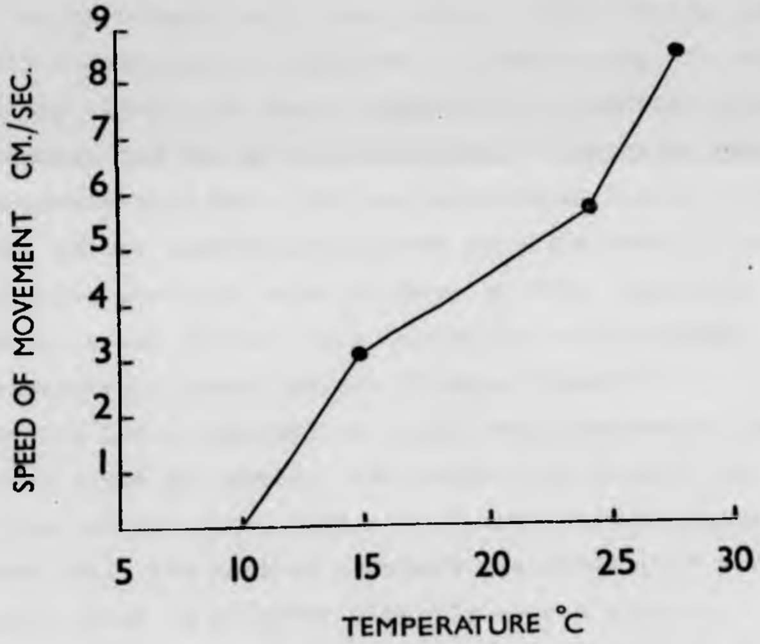


Figure 33. The influence of temperature on speed of movement in adult female Triatoma infestans. (Mean speed computed from data for three bugs).

10. The effect of ambient relative humidity on phototactic behaviour

(i) Introduction

The effect of relative humidity on the phototactic behaviour of triatomid bugs did not prove to be so readily or easily assessed as that of temperature and this environmental factor has not therefore been investigated here as thoroughly as it was hoped. First, there are fairly insurmountable problems in maintaining the relative humidity within the choice chamber at a constant level throughout the run of an experiment. A relative humidity of approximately 80% - 90% was maintained easily enough in the choice chamber but it was more difficult to achieve a relative humidity below a level of 30%. Initially this problem seemed to have been solved by using a Weyco Refrigerated Climate Cabinet (Fisons Scientific Apparatus Ltd., England) in which the experimental choice chamber could be placed. The cabinet is able to provide certain environmental conditions which may be easily controlled. The cabinet consists essentially of a working chamber which is situated directly over a special air-treatment system. Within this system the air is pre-treated so that it corresponds to the environmental conditions required by the experimenter. It was possible to control temperature levels within the cabinet fairly accurately but there were some problems in maintaining a constant relative humidity. A second drawback resulted from the considerable amount of noise and vibration inherent in the cabinet's motor mechanism. This constituted the most serious disadvantage in a behavioural study of this kind and so work with the cabinet was abandoned.

Attempts were therefore made to control the relative humidity within the central chamber of the polythene choice chamber by using solid potassium hydroxide (KOH). Potassium hydroxide is a white deliquescent solid which has found a useful application as a hygroscopic material in entomological experimentation. More usually, specially formulated solutions of potassium hydroxide are used for controlling humidity but lack of space did not make this a practical technique in the choice chamber. In any case, solutions of potassium hydroxide cannot achieve very low humidities, the lowest obtainable being in the region of 15%. However, since the air surrounding a solid piece of potassium hydroxide can have a relative humidity of as low as 3% - 4%, it was decided to fill the floor of the chamber with ground potassium hydroxide. This thin layer of potassium hydroxide was covered over with a sheet of filter paper so that the bugs had a normal surface on which to walk. At the same time, small muslin bags containing silica gel ($SiO_2.H_2O$) and potassium hydroxide were hung from the roof of the chamber in order to achieve a reduction of the atmospheric humidity in the chamber. Silica gel absorbs water vapour very readily and so also constitutes a useful hygroscopic material. The minimum relative humidity achieved using this technique was approximately 30% since it was difficult to obtain a reasonably airtight situation with the apparatus used. The drawbacks were incurred both in apparatus design and experimental procedure. As regards the latter, on returning bugs to the central chamber at each hourly observation it is difficult to obviate fluctuations in humidity during the transfer.

(ii) Testing the effect of relative humidity on the response to betalight in adult *Triatoma infestans*

In order to determine a possible effect of relative humidity, two choice chambers were used each equipped with a spherical green betalight of 2000 microlamberts (S10/G/2000). In apparatus A the temperature was 27.3°C. and the relative humidity was 29 ± 3 %. This humidity was achieved using the hygroscopic materials and procedure just described. In apparatus B the temperature was also 27.3°C. with a relative humidity of 90 ± 3 %. This humidity was achieved by omitting the hygroscopic material and at the same time hanging a piece of damp cotton wool wrapped in muslin from the roof of the central chamber. The experimental procedure used to determine a diel rhythm of attraction to betalight was followed and the experiment was run over three days. Adult female *Triatoma infestans*, post-emergence starved for 32 days, were used in this experiment.

The results are shown in Table 64. There appears to be no substantial difference in the response to betalight at either humidity tested. There is, in the peak period of response, a a difference in the mean percentage attraction of 2.2%, i.e. a slightly greater response at the lower humidity. However, it may be that humidities below 29% might have some effect on phototactic behaviour. The results of this investigation have been discussed in detail later in the thesis.

TABLE 64

The effect of relative humidity on the response to betalight in adult female Triatoma infestans

Apparatus A R.H.= 29.0± 3.0%

Time	B.T.	P.A.	B.T.	P.A.	B.T.	P.A.	Mean no. of bugs in betalight trap	Mean % Attn.
0700-1000	2	3.3%	2	3.3%	4	6.6%	2.66	4.43%
1000-1300	24	40.0%	25	41.6%	26	43.3%	25.00	41.66%
1300-1600	28	46.6%	22	36.6%	25	41.6%	25.00	41.66%
1600-1900	19	31.6%	22	36.6%	22	36.6%	21.00	35.00%
1900-2200	10	16.6%	11	18.3%	17	28.3%	12.66	21.10%
2200-0100	0	0.0%	0	0.0%	0	0.0%	00.00	0.00%
0100-0400	0	0.0%	0	0.0%	0	0.0%	00.00	0.00%
0400-0700	0	0.0%	0	0.0%	0	0.0%	00.00	0.00%
Totals	83		82		94		86.32	

Apparatus B R.H.= 90.0± 3.0%

Time	B.T.	P.A.	B.T.	P.A.	B.T.	P.A.	Mean no. of bugs in betalight trap	Mean % Attn.
0700-1000	2	3.3%	4	6.6%	1	1.6%	2.33	3.88%
1000-1300	33	55.0%	12	20.0%	26	43.3%	23.66	39.43%
1300-1600	24	40.0%	25	41.6%	19	31.6%	22.66	37.76%
1600-1900	17	28.3%	22	36.6%	17	28.3%	18.66	31.10%
1900-2200	7	11.6%	4	6.6%	9	15.0%	6.66	11.10%
2200-0100	0	0.0%	0	0.0%	0	0.0%	00.00	0.00%
0100-0400	0	0.0%	0	0.0%	0	0.0%	00.00	0.00%
0400-0700	0	0.0%	0	0.0%	0	0.0%	00.00	0.00%
Totals	83		67		72		73.97	

Abbreviations: B.T.= number of bugs in Betalight Trap
P.A.= Percentage Attraction

11. The combination of betalight and butyric acid as an attractant for triatomid bugs

(i) Attraction of 4th instar nymphs of Rhodnius prolixus to n-butyric acid

The aim of this experiment was to see whether triatomid bugs could be attracted to a source of n-butyric acid, $CH_3.CH_2.CH_2.COOH$. A glass choice chamber was used to study the bugs' responses to butyric acid. The chamber was not equipped with a betalight. One of the glass bottles of the chamber was filled with a small quantity of silica gel onto which a few drops of n-butyric acid was poured. The butyric acid was thus adsorbed by the silica gel and the acid could be detected as a distinct emanation throughout the twenty-four hour run of the experiment. In the control trap, at the opposite end, only a small quantity of silica gel was added to the glass bottle. A fine linen mesh screen was inserted between the ground glass connections of the lateral glass tube and the glass bottle of both the butyric acid trap and the control trap. This merely prevented the bugs from falling straight down onto the silica gel.

A diel rhythm of attraction to n-butyric acid was determined for 20 fourth instar nymphs of Rhodnius prolixus by observing the total number of bugs falling into the two traps at hourly intervals. After noting the total number of bugs entering the two traps at each hourly interval the bugs could be removed from the traps and re-introduced into the choice chamber via the glass introduction cylinder.

The detailed results shown in Table 65 below indicate a distinct diel rhythm of attraction by the bugs to a source of n-butyric acid. Mean values for the attraction of bugs to butyric acid are based on the results of three experiments.

The question of sensory adaptation was not investigated but it is obvious from the experiments that bugs will continue to respond to a source of butyric acid provided they are sufficiently active; bugs were photoperiodically entrained with a scotophase from 1300 - 1500 hours. Furthermore, once the bugs are removed from the butyric acid trap at each hourly interval, there is an opportunity for the bugs to re-gain their sensitivity to the stimulus.

In the presence of the butyric acid the antennae of the bugs can be seen to 'search' the air, presumably so that the olfactory receptors can locate the exact position of the acid vapour. It is also notable that the bugs appear to 'wipe' their antennae between the first pair of legs and that eventually the proboscis is fully extended. Once the chemical emanations from a host reach a sufficiently concentrated level then the proboscis is extended in preparation for obtaining the blood meal.

It is clear from other experimental work that the principal and probably only site of 'smell' or olfactory receptors in insects is on the antennae (Fraenkel and Gunn, 1961).

TABLE 65

Diel rhythm of attraction of 4th instar nymphs of Rhodnius prolixus to n-butyric acid

Time	Number of bugs in butyric acid trap (Mean)	Number of bugs in control trap (Mean)	Percentage attraction (Mean)
0700-1000	1	0	1.6
1000-1300	21	3.6	35.0
1300-1600	46.3	4.3	77.1
1600-1900	29.6	3.3	49.3
1900-2200	19.6	1.3	32.6
2200-0100	14.3	0	23.8
0100-0400	0.3	0	0.5
0400-0700	0	0	0

(ii) The combination of betalight and butyric acid as a suitable attractant for triatomid bugs

A distinct diel rhythm of attraction to n-butyric acid was clearly demonstrated in 4th instar nymphs of Rhodnius prolixus. The aim of the following experiments was to determine whether the addition of a second attractant, namely n-butyric acid, could significantly enhance the response to a betalight source.

In the first group of experiments (designated as Experiment A) the responses of third instar nymphs of Triatoma infestans were studied with respect to betalight, n-butyric acid, and a combination of betalight and n-butyric acid. In this investigation a glass choice chamber was used to study the responses of twenty bugs, post-emergence starved for 32 days. The bugs had been photoperiodically entrained with a scotophase from 1300-1500 hours. Three choice chambers were used: one chamber was equipped with only a spherical green betalight of 2000 microlamberts at one end, a second chamber with n-butyric acid only, and a third chamber with a combination of a spherical green betalight of 2000 microlamberts together with n-butyric acid. In each case the trap at the opposite end constituted a control and was not equipped with any attractant. The results are shown below in Table 66. Mean values are based on three experiments. There does not appear to be a great deal of difference in the relative attractiveness of each of the attractants although there is, perhaps as expected, a slightly greater response to the combined attractant. In each of these experiments there is something like maximum response obtained in ideal laboratory conditions.

Next it was decided to determine whether in the field bugs might show a significantly greater attraction to a betalight trap which was also provided with n-butyric acid. In these experiments (designated as Experiment B) the glass choice chamber was again used, this time with a combination of a betalight (S10/G/2000) and n-butyric acid in one trap and, concomitantly, a green spherical betalight (S10/G/2000) only in the trap at the opposite end.

The way in which 30 third instar nymphs of *Triatoma infestans* responded to the two groups of attractants was studied over a period of 24 hours. The bugs were post-emergence starved for 32 days. The experiment was repeated twice and the results presented in Table 66 are mean values based on three experiments. The total percentage response is a mean value based on a 9 hour period of activity and response.

The results indicate a significantly greater response to the combined attractant. It means that in those situations where bugs are presented with two kinds of trap, each of which is equipped with a betalight of the same colour and brightness, there is a real possibility that the bugs will select and be attracted to those traps also equipped with n-butyric acid, or indeed any other suitable chemical attractant. This finding may indeed have particular relevance in the field where all measures have to be taken to increase the size of the catch. The addition of a chemical attractant may enhance the overall trapping effect and thereby provide a more reasonable sample of bugs.

TABLE 66

Experiment A **The attraction of 3rd instar nymphs of Triatoma infestans to various kinds of attractant**

Time	Percentage attraction (mean values)		
	Butyric Acid	Betalight S10/G/2000	Butyric Acid and Betalight S10/G/2000
0700-1000	1	1.6	2.6
1000-1300	37.1	35.0	50.0
1300-1600	73.6	66.6	79.3
1600-1900	43.6	40.5	53.3
1900-2200	20.0	29.3	51.6
2200-0100	0	0	0
0100-0400	0	0	0
0400-0700	0	0	0

Experiment B

Time	Percentage attraction (mean values)	
	Betalight S10/G/2000 and butyric acid	Betalight S10/G/2000
0700-1000	7.3	2.5
1000-1300	42.2	9.2
1300-1600	42.5	17.7
1600-1900	32.8	15.1
1900-2200	24.4	8.1
2200-0100	4.4	0
0100-0400	0	0
0400-0700	0	0
Total percentage response (Mean)	39.2	12.8

DISCUSSION

Introduction

In the discussion which follows some aspects of the research pursued here in this investigation will be considered in detail with particular reference to the published literature.

Relation of light intensity to insect response

Experimental results obtained in this investigation have indicated that the brightness of the betalight is important in determining the magnitude of the phototactic response. In comparing the response to betalights of the same colour or wavelength but of different brightnesses it was noted that betalights of higher microlambert value attract significantly larger numbers of bugs. A betalight of greater brightness is therefore more attractive. This finding is indeed corroborated by other investigators (see review on light trap literature) who have used other light sources.

However, it would be as well at this stage to clarify the terminology used to describe the intensity of light sources. First, the term 'intensity' refers to the actual physical energy content of the photostimulus. The 'intensity' can be quantified either in terms of energy, e.g. watts, or numbers of quanta; alternatively, it may be defined in multiples of a given reference intensity. 'Brightness', however, is distinguished from the 'intensity' of a given stimulus and is measured subjectively in man or instead behaviourally by noting an animal's reaction. At any given wavelength the animal's reaction normally increases with 'intensity', the relationship being a logarithmic one in most cases. 'Brightness', therefore, as indicated by the simple magnitude of the animal's reaction, increases with the logarithm of intensity. It is interesting to note in this connection that the brightness of a betalight source is in fact measured according to the sensitivity of the human eye.

The difference between the terms 'intensity' and 'brightness' can in fact be explained in more complex physical terms but the simpler explanation seems adequate here.

The difference in terminology is clarified even further by the experimental results of Robinson and Robinson (1950). The Robinsons suggest that there are two properties of light which govern an insect's response to a light source. The first property is the power of the light source which is usually expressed in lumens. The second, in fact related to the first, is surface brightness which is expressed in lumens per sq. cm. More simply, surface brightness is the power per unit area of surface. It can be reasonably assumed from this definition that a light source with a high surface brightness is therefore one of high power and low surface area.

Having established these two different properties of light, the Robinsons then demonstrated a curious difference in the behaviour of insects to a light source according to physical variations in the two. First, an increase in the power of the light source resulted in an increase in the total number of insects taken in a trap.

Second, an increased surface brightness meant an increase in the catch of the total number of species. The Robinsons therefore concluded that for any power the optimum light source will be that with the greatest surface brightness.

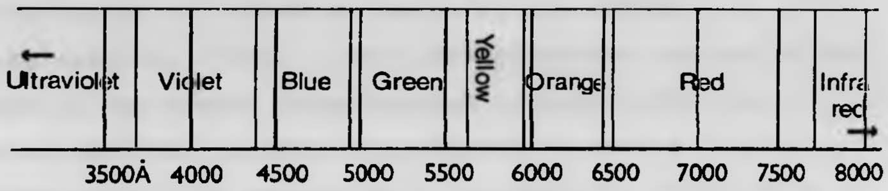
The factors of 'power of the light source' and 'surface brightness' are therefore of considerable importance to survey entomologists. A light source of high intensity can cause considerable inconvenience since the increased catch requires a great deal of sorting, the sorting being both tedious and time-consuming. The entomologist will therefore need to select a light source which will provide a representative sample of the prevailing insect population yet at the same time provide a catch which will not require endless sorting. On that score a light of high surface brightness and low power will perhaps be more suitable.

The whole question of brightness and intensity should therefore be considered carefully before embarking on any light-trapping procedure.

Wavelength Discrimination

The literature on the effectiveness of various wavelengths of radiation for stimulating responses in insects is voluminous and some consideration is given to it in the review of literature on light traps. Spectral response curves have been determined for a wide variety of adult and larval forms of insects and it has been found that insects as a class respond to radiation in the approximate range 2537-7000 Å, i.e. from the ultraviolet to the infra-red region. In general the effect of electromagnetic radiation is directly proportional to its absorption and photochemical effects which result in nerve impulses appear to be of primary importance in the region below 3500 Å.

Energy Spectrum



Two techniques are available to the entomologist for determining spectral sensitivity curves:

(i) Electroretinogram investigation.

This involves connecting electrodes near or directly to the optic nerves. The resulting potential developed when the eye is subjected to radiation can be observed on a recording oscilloscope. Spectral sensitivity curves can be obtained from the E.R.G. by relating the magnitude of potential to the wavelength of incident radiation.

(ii)Study of group motor responses to radiant energy.

Group motor response studies include both laboratory and field determinations of the relative effectiveness of the various regions of the spectrum. This is simply evaluated by noting the total number of a particular species which respond to radiant energy of a certain wavelength.

These two kinds of study have indicated that in general most insects show the greatest sensitivity to the near ultra-violet, then show a gradual decline to the blue, an increase to a secondary peak in the blue-blue-green region and finally the least attraction to the longer wavelengths of radiation (Weiss et al., 1942; Wigglesworth, 1964). This generalization applied in the case of the recent investigation of Snow (1971) who determined the spectral sensitivity of gravid female *Aedes aegypti* by noting their responses to oviposition sites illuminated by light of a defined intensity and spectral composition. It is also interesting to note that the honey-bee *Apis*, for example, does not respond to light of a wavelength greater than 6500 Å (Wigglesworth, 1964).

In order to assess whether an insect possesses the ability to discriminate colours it is necessary to determine whether it can distinguish between two visual stimuli of different wavelength composition but of the same intensity. However it must be stressed that even in the event of an animal not responding to a certain wavelength, colour discrimination should not be precluded since the experimental procedure may be at fault (Burkhardt, 1964). Indeed it appears that intensive study of the responses to a wide range of intensities and wavelengths with respect to any one insect under different environmental conditions is certainly necessary (Weiss et. al., 1943).

A preliminary investigation in this thesis has indicated that the colour of the betalight source is indeed of some consequence in the phototactic behaviour of triatomid bugs. The betalights used in the experiments on colour sensitivity were of different brightnesses and so an assumption that bugs do in fact distinguish between different wavelengths is perhaps a little suspect. Furthermore, the brightness as quoted for a betalight in the technical literature, is measured according to the sensitivity of the human eye and does not necessarily equate with the brightness as seen by other animals such as insects. It was hoped to have specially made betalights which would have approximately the same energy emission and which would therefore allow a precise evaluation of spectral sensitivity. Unfortunately, this does not resolve the problem entirely for although the quantity of energy which is incorporated in each betalight can be accurate to within ± 2%, there are nevertheless a number of additional factors which influence the total energy emission of a betalight and which cannot be determined by the manufacturers. Therefore, wavelength discrimination in triatomid bugs using betalight as a light source cannot be determined with any confidence.

Nevertheless, the results obtained in this study do indicate, perhaps tentatively, that triatomid bugs are much more sensitive to the blue-green region of the spectrum. The results also agree with the general finding for insects that the longer wavelengths of light are less attractive and here, consequently, the red betalight proved to be the least attractive. The data from this preliminary investigation are summarized below:

Betalight	Response expressed as a ratio
C135/B/90	1.000
C135/G/280	0.806
C135/Y/280	0.272
C135/R/40	0.200

Clearly, if it were only a matter of brightness then the yellow and green strip betalights might be expected to attract a much larger number of bugs than the blue. There is a possible argument that the bugs did in fact proceed to the low illumination blue light simply because it was the darkest of the situations available to them. However this may indeed be refuted since in choice chamber experiments designed to test the influence of brightness in which a chamber was equipped with two betalights of different brightness, there was a significantly greater attraction to the betalight of higher microlambert value. If indeed bugs were searching out the regions of lowest illumination then there would have been a larger number of bugs in the red betalight trap. This therefore suggests that bugs did not avoid the higher level of illumination of both the green and yellow betalight. Furthermore, in a second experiment it was shown that a green betalight of 280 microlamberts could attract a significantly larger number of bugs than a red betalight of a much higher microlambert value. If brightness were the sole factor involved, then the reverse situation might be expected. The results are useful in that they, at least give some indication of the type of betalight which might prove more successful for trapping bugs in the field, thus a blue betalight is more attractive than green and a green betalight proves more attractive than red.

In conclusion, it would seem that it is a combination of both the colour and intensity of a light source which will either initiate or fail to initiate a response, but it may be that of these two inseparable components, intensity is the most important since the longer wavelengths can be made equally or more attractive if their intensity is suitably increased (Weiss, 1946).

Orientation mechanism underlying the response of triatomid bugs to a betalight source

The way in which triatomid bugs orientate themselves to a betalight source must now be discussed adopting the terminology used by Fraenkel and Gunn (1961) in their classical descriptions of animal behaviour. Orientation mechanisms or, at least, descriptions of them, can be quite complex and so it is necessary at this stage to limit the discussion to a simple review of the ways in which bugs orientate to a light source, excluding the influence of other environmental factors.

First, Fraenkel and Gunn (1961) have divided the orientation mechanisms of animals into two broad groups, namely kineses and taxes. The transverse orientations also mentioned by Fraenkel and Gunn can be ignored in this study. Kineses have been described as undirected reactions. The animal reaches a source of stimulation as a result of random movements and there is certainly no orientation of the axis of the body with respect to the stimulus. In the second group of orientation mechanisms, called taxes, there is a directed reaction to the stimulus and a complex system of sense organs is required to make discrimination of direction possible. There is a distinct orientation of the axis of the body with respect to the stimulus. In klinotaxis, in which the insect will compare the intensity of stimulation in its sensory receptors by alternate movements to the left and right, the insect reaches the source of stimulation by regular alternating deviations and so its path is a very wavy one. In the two other examples of taxes, namely tropotaxis and telotaxis, the insect will follow a straight path either directly towards or directly away from the source of stimulation. In this investigation triatomid bugs were shown to be positively phototactic.

No detailed experimental study of orientation mechanisms in bugs was possible and so only a brief theoretical description is presented here. It would indeed have been interesting to follow the orientation of bugs to a betalight source on film which would then have allowed a more precise classification of their movements. Nevertheless in observations of their behaviour it became obvious that the bugs responded to the light source phototactically, in other words there was a directed reaction. This does not completely preclude the possibility of kinetic responses in triatomid behaviour since these may be exhibited by insects which not only have direction receptors but which also manifest taxes under certain conditions. Bugs may, for example, exhibit some kinetic response in their reaction to other stimuli. However, a taxis was indicated in the reaction to light and it is necessary to determine whether this was tropotaxis or telotaxis.

Initially, bugs were to be found in the darker regions of the choice chamber. As the bugs became progressively more active there was a gradual movement of bugs out into the centre of the chamber, so that they aligned themselves along the beam of light. The author noted a slow deliberate movement out from the sides with a gradual 'swing' in the orientation of the bugs towards the betalight end of the chamber. Bertram (personal communication) in initial observations of <u>Triatoma</u> <u>maculata</u> also gained the impression that the overall rate of approach to the light was slow with long spells of bugs remaining stationary while facing in the direction of the light. A gradual curve round towards the light source with long periods of remaining stationary might be explained in terms of tropotaxis. In a tropotactic orientation there must be at least two receptors which are so arranged that they are not always equally stimulated. The compound eyes certainly seem to fulfil this condition since it would be possible for only one eye to be stimulated if a bug should align itself at right angles to a beam of light. If tropotaxis is indeed the operative mechanism then the following description would apply. A bug moves out towards the beam of light in such a way that there is asymmetrical stimulation of the compound eyes. This would lead to a turning reflex such that the bug turns gradually to the illuminated side. The turning continues until the two eyes are equally stimulated which means that the bug will eventually turn towards the light. When the stimulation becomes symmetrical the turning ceases and the bug moves straight forward towards the light. In fact once there is equality of stimulation in the two compound eyes, the two sets of impulses are 'cancelled out' in the central nervous system and so turning stops. An essential feature of tropotaxis is simultaneous comparison of light intensities on the two sides and an orientation according to the resulting balance.

Tropotaxis would seem to be the probable orientation mechanism. No unilateral blinding experiments were performed on the triatomid bugs. This kind of experiment can help the investigator distinguish between tropotaxis and telotaxis. In tropotaxis it is often found that the insect can no longer orientate properly when the photoreceptors of one side are eliminated. However, in telotaxis orientation in the direction of the source of stimulation occurs without balance. The insect 'fixates' the source of stimulus with its sense organs and advances towards it in such a way that only a certain region of the receptor apparatus is continuously subjected to the stimulus. In a further experiment to distinguish between the two types of reaction, the insect may be subjected to two light sources; in tropotaxis there is a distinct orientation between the two lights whereas in telotaxis the insect will proceed to only one of the lights and ignore the second.

Experimentation along these lines is recommended for triatomid bugs to resolve the mechanism although it must be remembered that the two mechanisms may indeed work together, as has been demonstrated in the bee (Fraenkel and Gunn, 1961) which initially shows a tropotactic response to a light source.

In those experiments involving a study of temperature and its effect on response to a betalight source, an orthokinetic mechanism may indeed be important. A variation in linear velocity is called orthokinesis and the average speed of movement or frequency of activity depends on the intensity of stimulus. It may be that orthokinesis simply results in greater random movement which means that the next orientation mechanism, namely tropotaxis or telotaxis, may consequently be speeded up. It is important to remember that orthokinesis, being an example of an undirected reaction, is rather inefficient as an aggregating mechanism.

Finally, the question of sensory adaptation needs to be discussed. A change in the excitability of a sense organ as a result of continuous stimulation such that a more intense stimulus becomes necessary to produce the same response is called sensory adaptation. After a period of illumination the eye of an insect is said to be light adapted and it becomes progressively less sensitive. On return to the dark, however, the eye gradually resumes its sensitivity as it becomes dark adapted until eventually a maximum sensitivity is attained. Adaptation to light is thought to result partly from the movements of pigments in the ommatidia, partly from a neurological change in the brain itself and partly from physico-chemical changes in the receptor mechanism of the retina.

It will be remembered that in many of the investigations of phototactic behaviour the bugs were exposed to a betalight source over a period of twenty-four hours. It might be expected that over such a long period of exposure there should be some sensory adaptation to the light source. However, the constant exposure was found not to influence the bugs' level of phototactic behaviour and, indeed, the bugs would respond to betalight according to their photoperiodic entrainment. Generally, there was a good response to the light source over a period of 9 hours or so. The gradual decline in response on either side of the period corresponding to the scotophase of the entrainment is to be expected since activity begins to decline. Furthermore, in the period of inactivity the bugs were shown to accumulate along the sides of the choice chamber, the darker regions of the chamber, thereby eliminating excessive exposure to light. Once the bugs do emerge into the brighter regions of the chamber, respond to the light, and fall into the trap, it is possible that their eyes have attained the light adapted state. However, on falling into the darker regions of the trap (see Figure 1) there is the possibility that during the hour between observations the eyes may revert gradually to the dark adapted state, in which case the eyes would once again be sensitive to the betalight. The longer the period of adaptation to darkness the more rapidly the insect will respond when exposed suddenly to a light of given intensity (Wigglesworth, 1965). In this study the use of a rather subdued form of illumination, in the form of betalight, should preclude the possibility of a very long period of dark adaptation. On that basis, one hour should be sufficient for the compound eyes to resume their sensitivity to the light source. The fact that the bugs did continue to respond to a light source after an interval of an hour meant that they had achieved the dark adapted state and that they were sufficiently sensitive to respond to this light of low intensity (Chapman, 1969). The whole question of sensory adaptation certainly warrants further experimentation and it is suggested that this phenomenon may indeed have to be viewed in a slightly different context in field conditions.

Finally, something must be said of the role of the ocelli in sensory adaptation and the orientation mechanism. Dark adaptation in the dorsal ocelli of insects is discussed by Goodman (1970) and it would seem that this state may be achieved within a remarkably short space of time. There is, therefore, no reason to suppose that the ocelli in triatomid bugs will differ remarkably in their adaptation from that of the compound eye or indeed that they might in some way interfere with the course of adaptation. Furthermore, the ocelli in bugs, shown in Figures 12 and 13, may also, as a result of their position, be subject to unequal stimulation and so would not preclude the possibility of a tropotactic orientation.

The role of the ocellus in triatomid bugs

Before considering a possible role for the ocelli in triatomid bugs it would be useful to review the work carried out on the structure and function of the ocellus in other insect groups. This may at least help formulate theoretical considerations with reference to triatomid bugs.

Many insects possess two types of eye: a complex faceted compound eye to which can be ascribed the powers of vision, namely the perception of form and colour and appreciation of movement in the visual field; and simple eyes or ocelli. Ocelli are simple cup-shaped eyes situated in the dorsal region of the head. The occurrence of the ocelli within the Insecta appears to be extremely erratic and does not bear any apparent relation to the mode of life or the systematic position of those insects in which it occurs. Furthermore there are no very obvious differences in behaviour between ocellate and anocellate species (Goodman, 1970).

The general structure of the ocelli in insects has been reviewed in considerable detail by Goodman (1970) and Mazokhin-Porshnyakov (1969) and so this need not be discussed in too much detail here. Basically an ocellus consists of a few hundred visual cells situated beneath a common lens. The visual cells are innervated directly from the ocellar lobes which are located in the protocerebrum. The organization of the dorsal ocelli is different from that of the compound eyes yet Mazokhin-Porshnyakov (1969) states that they have the three necessary components for vision, namely, photorefracting, photosensitive and photoinsulating devices. Certainly this is description enough for there is some organizational and functional variation in the ocelli of different insect groups and therefore it would be unwise to carry the generalization any further. Indeed there does not appear to have been any extensive investigation of the structural organization of visual sense organs in the Triatominae.

However, it does seem certain that for ocelli in general the optical system of the organ is such that no image can be formed on the retina. The close proximity of lens and retina therefore precludes any possibility of image formation.

What then is the role of the ocellus in insect behaviour? Entomologists are still a long way from understanding the full role of the ocelli as photoreceptors. What is clear is that the ocelli are equipped with photosensitive cells which are not functional in form vision. Indeed the ocelli are not physiologically equivalent to the compound eyes and certainly cannot replace these organs of vision. The ocelli merely supplement the compound eyes.

In triatomid bugs the ocelli have been shown to be involved in the phototactic response to betalight for adult bugs whose compound eyes have been painted over still respond to the light source. The nymphs of triatomid bugs however do not possess ocelli and so when their compound eyes are painted over the bugs are totally blind and do not respond to the betalight source. Does the implication of ocelli in the phototactic response to betalight agree with the findings of other investigators studying the ocelli in other insect groups?

In a simple phototactic response to betalight no complicated image formation is necessary and so the fact that the optical system of the ocellus precludes this does not really matter. It is also stated that the ocelli have indicated sensitivity to a wide spectral range and that they therefore possess colour vision (Mazokhin-Porshnyakov, 1969). This would suggest that the ocelli might respond to light sources of a wide wavelength range. Certainly the ocelli respond to green betalight, S10/G/2000.

Parry (1947) studied the electrical responses in the nerves of Locusta migratoria. Darkening the ocellus was found to cause a depolarization which spread down the ocellar nerve, finally depolarizing a ganglion in the brain and inducing a discharge of impulses down the commissures. As soon as the ocellus was reilluminated the discharge of nerve impulses in the commissures ceased. It is therefore suggested that from an electrophysiological point of view the ocelli serve not only to signal light changes but also serve in the perception of light as opposed to darkness. Because the frequency of impulse activity in the nerves bears a direct relation to the level of illumination it is also suggested that the ocelli can therefore supply information on the absolute luminosity. On the basis of his work, Parry (1947) suggests that the ocelli are not capable of evoking a motor response by themselves but only do so when they work in cooperation with the compound eyes. It may then be that the ocelli in some way 'prepare' the nervous system for reception of light stimuli. Ultimately the ocelli may in some way accelerate the animal's response to shadows.

The implication is therefore that the ocelli are only stimulatory organs which can increase the response to stimuli received through the compound eyes, but do not in themselves evoke a motor response. Experimental work that confirms this view includes that in which Drosophila were shown to react more rapidly to a sudden exposure of light with ocelli intact than when blinded, and that in which Calliphora and Locusta manifested an inability to orient themselves in relation to a light source when the compound eyes are blinded and the ocelli left intact (Cornwell, 1955). These findings are perhaps contradictory to those obtained here in which adult triatomid bugs show a distinct phototactic response to betalight even if the compound eyes are blinded, therefore implicating the ocelli in a motor response. However, the findings do agree with those of Wellington (1953) whose work on the ocelli of the dipterous parasite Sarcophaga aldrichi Parker., has shown that the ocelli are in themselves quite capable of evoking motor responses, and that a fly with only ocelli operative can still make directed movements and therefore orient itself.

Perhaps the divergence of opinion necessitates a reasonable compromise. Wigglesworth (1965) has rightly suggested that adult ocelli vary considerably in complexity and that it may be foolhardy to expect function to necessarily correspond in all insect groups.

In conclusion, it is difficult to ascribe a definite function for ocelli in triatomid bugs. The ocelli certainly respond to betalight in the laboratory situation, but what is their more usual role in natural circumstances? Perhaps as suggested by Parry (1947) the ocelli supply information on the absolute luminosity of the environment. Alternatively the periodic changes in the natural light and dark cycle which may initiate locomotory rhythm in triatomid bugs perhaps operates not only through the compound eyes but also the ocelli (Cloudsley-Thompson, 1961).

Clearly there is need for further research along these lines.

The importance of photoperiodism

The important role that photoperiodic entrainment can play in determining the onset and duration of locomotor activity in triatomid bugs has already been emphasised. It has been indicated in the results obtained here that the onset and duration of attraction to a light source will correspond to the onset and duration of locomotor activity. Clearly then a definite cycle of light and dark will govern an insect's total behaviour very precisely and so the whole question of photoperiodism will be briefly considered here.

The environmental rhythm of recurring alternation of illumination and darkness is the earth's natural photoperiod. The diel rhythm of photoperiod has not only a profound influence on the distribution, seasonal biology, growth, metabolism, and behaviour of animal organisms, but also influences the temporal organization of the internal processes. It is also important here to gain some concept of time for the endogenous rhythm of the functional organization of the animal is influenced by the exogenous rhythm of the environmental cycle of light and dark. Furthermore, photoperiodism can be distinguished from the more direct responses of the organism to light since the influence of photoperiod is most frequently in the form of time-dependent stimuli, e.g. the beginning of daylight and the beginning of night, rather than in the form of a continuous input of light energy.

Daily patterns of behaviour are apparent in the activities of most insect species. Locomotion, feeding, mating and oviposition are behavioural phenomena that may occur at species-typical times of the day. Many insects are typically active during daylight hours (diurnal species), others tend to be active at night (nocturnal), while others are active mainly during the evening or morning twilight (crepuscular). The insect may be specially adapted to live at a specific time of day and so its entire biology will be geared to that period.

The distinct diel rhythms of activity which are apparent in the Insecta have been extensively reviewed by Cloudsley-Thompson (1961). Corbet (1960) considers that physical factors of the environment can certainly influence or modify the patterns of cyclical activity in insects, particularly exemplified in the biting cycles of the Culicidae living in forests. However the timing of the activity must be determined by some suitable time cue (or zeitgeber) on a day to day basis and of the extrinsic factors available the one most likely to fulfil this role is light. In general then, it seems that the role of light is to stimulate or release activity. The activity can only become manifest however if other factors such as humidity, temperature and wind provide a microclimate suitable for its expression.

Clements (1963) believes that the time cues provided by a natural cycle of light and dark will ultimately control the various activities of mosquitoes such as biting, swarming and oviposition. Certainly the influence of photoperiod on oviposition has been investigated in some detail. For example, mosquitoes which have been kept throughout life in constant dark oviposit irregularly but when they are transferred to a regime of 12 hours of light followed by 12 hours of dark per day they immediately start to oviposit regularly and the rhythm is maintained for at least 3 days after return to constant darkness, temperature and humidity, suggesting that it is circadian in nature (Gillett et. al., 1959; Haddow et. al., 1961; Haddow and Gillett, 1957, 1958). The investigations have also indicated that single exposures to light in an otherwise all-dark existence initiate regular cyclic oviposition. The study has also shown that it is in fact the return to darkness which constitutes the time-cue.

Various investigations of the biting cycles of mosquitoes have been undertaken and here too a distinct periodicity is obvious. Twenty-four hour catches have been set up in order to determine the rhythmic biting cycles of various species of mosquito (Haddow, 1956; Haddow and Ssenkubuge, 1965; Lumsden, 1955, 1956, 1957; Reuben, 1971a). Indeed Haddow (1954) believes that the 24-hour catch is probably the most valuable method of studying biting behaviour.

It is interesting to note that the diel rhythm of activity is very much influenced by various environmental factors such as microclimate (Haddow, 1945; Lumsden, 1956; Cloudsley-Thompson, 1961). For example, the peak of biting activity of _Mansonia_ _fuscopennata_ occurs at dusk at all levels in the forest but in a banana plantation surrounded by forest it occurs several hours after dusk at a time when the microclimatic differences between forest and plantation have disappeared (Clements, 1963).

The physiological condition or age of the insect can also modify its diel pattern of activity (Corbet, 1960).

The diurnal periodicity of flight by insects is considered in relation to photoperiod together with the important influence of environmental factors by Lewis and Taylor (1964). Photoperiod is the major factor controlling the precise time of flight and this will of course change seasonally with the time of sunrise and sunset.

Biting activity curves of mosquitoes are usually curves of great complexity and great variability simply as a result of the tremendous variation from day to day, the variations from forest level to forest level, and even as a result of the differences in patterns of flight activity of males and females (Lumsden, 1965).

Mating behaviour is also subject to photoperiodic entrainment. The Mexican fruit fly can be observed to mate during the late afternoon and early evening. When the photoperiod is experimentally reversed the daily mating activity is also reversed suggesting that the copulatory rhythm can be photoperiodically entrained (Flitters, 1964).

The stridulatory activity of the orthopteran _Eugaster_ appears to follow a diel rhythm (Nielsen, 1971). The glowing of the Lampyridae also has a diel nature (Dreisig, 1971) and here again photoperiodic entrainment seems to be important.

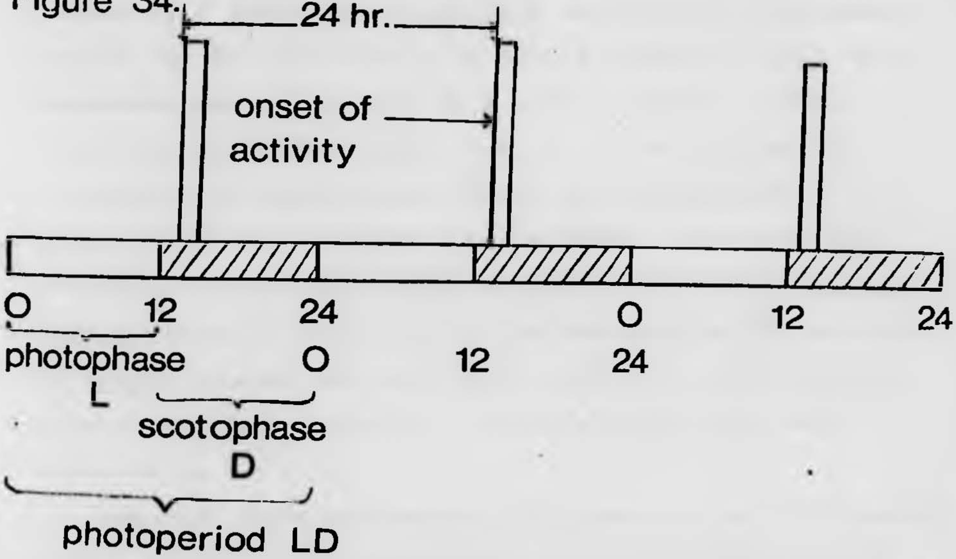
General locomotor activity can also be photoperiodically entrained as Dreisig (1971a) demonstrated in the case of the dusky cockroach, Ectobius lapponicus. In the cockroach Blatta orientalis, there is a distinct rhythm of activity under normal conditions of light and darkness. This takes the form of a burst of activity in the early part of the night, mainly before midnight, and a quiescent period in the later part of the night and during the day. This rhythm can be eventually eliminated by keeping the insects in constant light or darkness (Harker, 1954). A diurnal rhythm of activity was determined for mayfly nymphs and here it is interesting to note that the rhythm is not seen in nymphs bred from eggs which have been kept in continuous light but will appear abruptly after exposure of the nymphs to natural daylight and darkness for a period of no more than 24 hours (Harker, 1953).

Insects have exploited the phenomenon of photoperiod in their evolution of ecological, physiological, morphological and behavioural adaptations. Clearly the Triatominae have evolved so that their ecological, physiological and behavioural adaptations require a nocturnal habit. The nocturnal habit does, interestingly enough, confer a number of advantages; enemies are easily avioded; food can be more easily obtained; losses of moisture are considerably reduced; and odours are more easily conveyed so that blood-sucking insects such as the Triatominae can locate their prey (Cloudsley-Thompson, 1961). Clearly then, the function of photoperiod in these biological adaptations is to provide environmental information in the form of temporally spaced signals (e.g. dawn and dusk) to which the insects can respond in diverse ways. Photoperiod clearly influences the activity patterns of triatomids in the laboratory situation but it would indeed be interesting to determine if this is so in the field. There must be some zeitgeber to control the behavioural patterns of triatomids and of all possible zeitgebers, light seems the most probable.

Phase-setting and Terminology

Figure 34 below shows some of the basic characteristics of the relationship between the insect's endogenous behavioural rhythm and the exogenous photoperiod. In figure 34 it will be seen that activity begins very shortly after dark in each cycle. When the activity is maintained in a constant time relationship with respect to photoperiod the behavioural rhythm is said to be photoperiodically entrained. The time-cues or zeitgebers provided by photoperiod are necessary for the daily adjustment or phase-setting of the behavioural rhythm. If the insect is not subjected to this daily photoperiodic entrainment then the endogenous behavioural rhythm can be described as 'free-running'. The experiment to demonstrate the presence of a biological clock, described

Figure 34.



here in this investigation, indicated a 'free-running' rhythm. 'Free-running' rhythms, as shown in that experiment, still manifest a distinct periodicity which however may only be approximately 24 hours and therefore described as circadian.

Initiation of the rhythm in the insect introduces another interesting aspect of photoperiodism. A rhythm can be initiated in a totally arrhythmic insect by subjecting it to a short unrepeated light period. However, whereas in the cockroach the duration of the light period necessary to establish the rhythm is in the region of 2 hours, in Drosophila an eclosion (emergence) rhythm can be initiated by a single flash of light which approximates to 1/2000th of a second (Harker, 1961). Furthermore, Muller (1965) has indicated that by the alteration of the precise timing and duration of photoperiod it is possible to influence the onset and duration of the behavioural rhythm. Muller established the importance of photoperiod in determining the activity of Baetis nymphs and found that even after artificially shortening their 'nights', activity could still be observed in LD 23:1.

However, notwithstanding the importance of photoperiod, under natural field conditions insect activity will be influenced by many other factors which will strongly regulate the manifestation of the insect's behavioural patterns. Some of these factors have been considered here in this investigation.

A Biological Clock in the Triatominae?

Having considered the implications of photoperiodism, it is then necessary to formulate some concept of a time-measuring system within the functional organization of the animal and such a system has been termed a biological clock. There has been much debate over the use of the word 'clock' but Pittendrigh and Bruce (1959) have decided that it is appropriate in the context in which it is used. Indeed Lumsden (1965) has said that insects may be endowed with a physiological clock which adjusts some of their activities to an approximately 24-hour cycle in relation to some starting stimulus.

Insects display individual and population behavioural patterns that occur on a predictable daily basis and these behavioural rhythms will usually continue to be manifested when the insects are experimentally isolated from obvious environmental stimuli such as light. Indeed the investigation here has shown that triatomid bugs will manifest a distinct circadian rhythm of attraction to a betalight source over a period of several days in the complete absence of a zeitgeber. This must inevitably suggest that bugs possess an innate capacity to measure the passage of time. The curious thing about the 'clock' in insects is that it is regulated by exogenous factors but can function without regulation for a period varying in different groups from a few days as in cockroaches to months or longer as in the case of some beetles (Harker, 1956).

Although no full experimental investigation of the possible nature of the biological clock in triatomid bugs has been attempted here, nevertheless a full theoretical consideration will be given to the nature of the clock since there is evidence to suggest the presence of such an intricate piece of biological chronometry in bugs and therefore the concepts discussed here may be relevant.

Brady (1969) has put forward some interesting theoretical concepts concerning the nature and possible location of a biological clock in cockroaches. The first question that needs to be asked is "Does a localized anatomically identifiable mechanism control the timing of the rhythm?" Harker (1954, 1955 and 1956) believed that the rhythm of one cockroach could be transferred to another by simply connecting their blood systems. This proved to be a misconception. Harker (1956) also claimed that implantation of the suboesophageal ganglion from a cockroach with a known rhythm into a totally arrhythmic cockroach would result in the appearance of the same rhythm in the arrhythmic cockroach. Later it was suggested that implantation of the ganglion would not transfer the rhythm if two pairs of lateral neurosecretory cells were removed from the ganglion. Furthermore, cutting the nerves that connect the corpora cardiaca, source of the neurosecretory material, to the suboesophageal ganglion, was said to cause a loss of rhythmicity. These concepts proved on further experimental evidence cited by Brady (1969) to be wrong.

Where then is a possible location for the clock? The compound eyes are the pathway for the most important environmental zeitgeber, i.e. light, and this information must be passed through the optic lobes to reach the central nervous system. Experiments have shown that in the cockroach at least, if a cut is made between the optic lobes and the rest of the brain, the insect will become arrhythmic. The experimental evidence therefore suggests the existence of some kind of circadian pacemaker in the optic lobes. Finally, if a transverse cut is made through the circumoesophageal connectives of the brain in cockroaches this also results in the insect becoming arrhythmic. This suggests that the pacemaker in the optic lobes asserts its effect on the thoracic ganglia electrically through normal neural pathways in the ventral nerve cord. It would indeed be interesting to see whether the same mechanism holds true for triatomid bugs.

It is only possible at this stage to assert the presence of an innate time-measuring device in triatomids.

Physiological considerations

Apart from all those exogenous factors which may influence the operation of a light trap in the field, the physiological condition of the insect may also have some significance in determining the relative success of the trap catch. The evidence from this study suggests that nutritional condition is significant both in the adults and immature stages of triatomid bugs. A phototactic response of considerably greater magnitude is evidenced in those bugs which have fed some time previously. This finding is corroborated by Sjogren and Ryckman (1966) who found that the nocturnal flights of Triatoma protracta as indicated by collection in black light traps usually occur when the bugs are in a starved condition and also when they are stimulated to fly by above average summer temperatures. The study of Wood and Anderson (1965) further indicates that bugs will usually tend to invade human dwellings if there is an absence of suitable rodent hosts as a food source, and if they are subject to temperature stress. Interestingly, Wood (1943), while searching for Triatoma longipes in homes in a mining district of Arizona, U.S.A., discovered that all the adult bugs collected by him had fed some time previous.

The way in which the nutritional condition may influence the activity of a triatomid bug will be considered theoretically. Directly after a blood meal adult triatomid bugs manifest considerable inactivity, perhaps in this early stage the physical constraints conferred by a somewhat distended abdomen are important in limiting activity. Defaecation results during or a short while after the feed so that some of the abdominal volume is reduced, there then being much less of a constraint on activity. Activity increases such that the bugs are able to return to their crevices to digest their blood meal. As time progresses so the blood meal is gradually digested and thus the end-products of digestion become available as sources of energy which may be utilized for general locomotor activity. Wiesinger (1956) describes this period of digestion as the 'rest period'. Adult bugs do in fact ingest a large quantity of blood which is digested at an unusually slow rate. It may be that the progressive increase in activity levels of triatomid bugs, with increasing number of days after a blood meal, may in fact reflect this gradual digestive process, which in turn suggests that only around 16 days or so after a feed, as shown experimentally in this investigation, are sufficient energy-rich products becoming available to the muscles to achieve something like maximum locomotor activity, and hence, maximum phototactic response. This is certainly one possible explanation. Alternatively, the enhanced levels of activity may simply reflect a considerably depleted level of metabolic reserves and hence a fairly starved condition. Adopting this second theory it might be supposed that the blood meal has been completely digested and that the breakdown products of digestion have been utilized. A bug would become progressively more active as it used up its nutritional reserves. In that case a bug would need to become active to locate its next blood meal.

At any rate, having attained something like peak levels of activity at or around 16 days, this level of activity is continued for some time thereafter, according to levels of metabolic reserves, so that the bug's chances of locating and obtaining a second blood meal are thereby enhanced. Wiesinger (1956) has indicated a period of three weeks between blood meals and has stated that in the last nights before the next blood meal there is a particularly marked increase in activity. This finding agrees with the observation noted here of enhanced levels of activity and response at 16 days or so following a blood meal. However, it is important to note that it does not seem to be essential for bugs to obtain a second feed immediately since they can resist several months of starvation.

In the case of immature bugs, despite a proportionately larger blood meal, there is fairly marked activity following the meal, certainly a much greater level of activity than is evidenced in adults after their feed. Nevertheless, digestion over a period of two weeks or so appears to be essential before the bugs' phototactic responses start to approach maximum level.

In brief, some considerable time must elapse after the blood meal before activity is sufficiently marked for adult and immature bugs to become attracted to a light source in reasonably large numbers. Nutritional condition is therefore the kind of factor which will determine whether or not a light trap has the potential to operate successfully in the field (U.S. Department of Agriculture, 1961).

The way in which bugs respond to a betalight source following emergence was also studied. There is, in the absence of a blood meal, a progressive increase in the magnitude of the phototactic responses of adult <u>Triatoma infestans</u> and adult <u>Triatoma maculata</u> with gradually increasing number of days following their emergence from the 5th instar. A similar effect was observed for 5th instar nymphs of Triatoma phyllosoma, starved since emergence from the 4th instar. The nymphs' phototactic responses manifested a gradual increase over a period of 40 days. This group of experiments seem to be corroborated by those of Brady (1972) who has shown a steady increase in activity with advancing age and starvation in teneral males of Glossina. Brady suggests that 'ignoring circadian and other fluctuations, the average daily intensity of activity is a measure of the nutritional state of the fly, and that some feature of the metabolic reserves may therefore ultimately control overall activity level.'

Moulting also has significance in that during the period preceding the moult the bug's phototactic behaviour is very distinctive. Experiments have shown that as the bug prepares to moult so the response to a betalight source lessens progressively. The actual moult or ecdysis renders the bug virtually inactive so that it cannot respond to a betalight source. It may be that during the period prior to the moult, metabolic reserves are diverted specifically for metamorphosis, there not being sufficient reserves for general locomotor activity. This would then certainly explain the decline in activity and hence phototactic response during the period prior to the moult.

The whole question of nutritional condition clearly warrants further investigation, including a thorough biochemical study to resolve the ways in which the blood meal is digested and the manner in which its digested end-products are utilized in metabolism.

The influence of temperature on the behavioural patterns of triatomid bugs

Useful and informative reviews discussing the important influence of temperature in the lives of insects are presented by Chapman (1969) and Wigglesworth (1965). These provide an invaluable theoretical background to an understanding of the way in which this environmental factor can affect triatomid behaviour.

The temperature of the air is of particular importance to an insect since its own body temperature will approximate very closely to it. Changes in air temperature have therefore been shown to have an immediate effect on digestion, excretion, movement, reproduction, development and various other activities. In this investigation the influence of temperature on the general activity of the bug has been considered in some detail for ultimately, as the results obtained in the experimental work suggest, the bug's general level of activity will determine whether or not it will move towards a light source and hence the relative attractiveness of that light source.

Weather and climate are commonly accepted by entomologists as dominant influences on the behaviour, abundance and distribution of insects. Of the climatic range of factors, temperature is considered perhaps the most important and indeed has constituted the basis of many physiological investigations (Messenger, 1959). A great many of these investigations have been aimed at determining temperature thresholds, optimal thermal conditions and lethal levels but ultimately as suggested by Wellington (1957) there is a real need to see how temperature will influence insect behaviour.

Before reviewing the effects of temperature on insect behaviour it is necessary to say something about the upper and lower limits of ambient temperature acceptable to the Insecta. Generally, insects will die if exposed to a temperature of 40°C. to 45°C. (104° to 113°F) for one hour (Buxton, 1933). A few species can of course survive much higher temperatures but the above figures will apply to a great majority of the insect groups. It is perhaps more difficult to state categorically a definite lower limit for it is generally true that insects can survive temperatures many degrees below that which they encounter in nature. Some insects can for example survive prolonged exposure to extreme cold such that activity is limited by the unfavourable temperature but will resume on return to a more favourable environment.

Because there is such a close correlation between the body temperature of the insect and the ambient temperature it can be appreciated how dramatically this factor can affect the rate of metabolic processes in the organism and hence its general activity. The activity of the desert locust, <u>Schistocerca gregaria</u>, for example, has been shown to be governed very precisely by temperature, for a state of cold stupor results below 17°C. The locust will only begin to resume activity between 17°C. and 20°C. (Fraenkel and Gunn, 1961).

It is known that most terrestrial poikilotherms will enter a state of torpor at fairly low temperatures, somewhere between freezing point and 15°C. Nicholson (1934) has studied the locomotor activity of the blowfly, Lucilia cuprina in relation to temperature and his research has shown a close correlation between the two. Nicholson simply derived his correlation by estimating the proportion of animals which moved at each temperature and ignored the whole question of velocity. He found that activity increased from zero at 5°C. to a first maximum at 20°C., remained steady to 30°C. and then fell slightly to 35°C. before rising steeply to a second maximum at 42°C. Above that temperature there was a rapid decline in locomotor activity and lethal effects became obvious. Nicholson interprets the high temperature peak of activity as an orthokinetic response to temperature, a response which would ensure that the organism did not remain long in the unfavourable temperature range.

Mellanby (1936) found a similar correlation in his experimental investigation of the tsetse fly, Glossina palpalis. Flies were not particularly active at temperatures below 21°C. (Triatomid bugs also manifested steadily decreasing levels of activity at temperatures below 21°C.) Furthermore, below 8°C. tsetse flies were completely immobilised.

A similar pattern of behaviour has been observed in the case of triatomid bugs here in this investigation. The results obtained from the experimental work have revealed that the phototactic response to betalight shows a progressive increase with each gradual rise in temperature. Furthermore, it has been shown that the response to a light source must ultimately depend on locomotor activity. Since temperature has been shown to influence activity levels, bugs being completely inactive at or around 8°C., activity being resumed with a gradual increase in temperature, a distinct correlation can be drawn between the relative attractiveness of a light source and ambient temperature.

Temperature has been shown conclusively to exert an important influence on the speed of locomotion of an insect. The influence of temperature on the rate of progression in *Periplaneta americana* has been investigated by McConnell and Richards (1955) and their research indicates that the cockroach will run significantly faster at higher temperatures. Their work has shown that the speed of cockroach movements is related to temperature as depicted by the Arrhenius relationship; so that a plot of the natural logarithm against the reciprocal of the absolute temperature gives a straight line.

Thermokinetic studies on ants, *Thiometopum apiculatum*, are described by Shapley (1920 and 1924). Shapley set up a very simple experiment in which he measured the rate of locomotion of a trail of ants at varying temperatures. His results indicate that as the temperature rises 30°C. the speed changes 15-fold, increasing uniformly from 0.44 cm./sec. to 6.60 cm./sec. It also appears from Shapley's work that other meteorological factors, with the possible exception of rain, have very little if any effect on the kinetic response. So constant was the temperature to speed of locomotion relationship that Shapley could estimate the temperature to within 1°C. from a single measurement of ant speed.

The speed of locomotion of triatomid bugs also appears to be strongly influenced by temperature for experimental results obtained suggest that bugs will move considerably faster at higher temperatures. This may indeed be an example of an orthokinetic reaction. The reason for this increased rate of locomotion has not been investigated here but Shapley (1920) suggests that variation in the activity of the poikilotherm is largely governed by the metabolic processes of the organism which in turn will depend on the rate of oxidation and various other internal chemical reactions. The physical factor of temperature will affect these chemical processes and therefore it seems reasonable to postulate that it should also influence the kinetic responses of the organism. Indeed Crozier (1924) investigating the velocity of progression of a diplopod, has stated that "in common with many chemical reactions, at ordinary temperatures, the velocities of a variety of protoplasmic activities are found to be about doubled or trebled by a 10° rise of temperature." Crozier therefore suggests that the rate of arthropod locomotion must ultimately depend on the central nervous system, for temperature may directly affect the rate of discharge of nerve impulses from the nerve centres.

Perhaps another mechanism may be operative to explain response. So constant was the temperature to speed of locomotion relationship that Shapley could estimate the temperature to within 1°C. from a single measurement of ant speed.

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Perhaps another mechanism may be operative to explain the increased rate of locomotion. Chapman (1959) has shown that the body temperature of an insect is of overriding importance in limiting flight and that all insects have a minimum body temperature below which flight is quite impossible. The important factor here lies in muscle physiology. The length of time taken for the completion of a flight muscle twitch is so long at temperatures below 24°C. that flight of Schistocerca is inefficient and the twitch is only sufficiently short above 30°C. for really sustained flight to occur. The wingbeat frequency of Drosophila is also influenced by temperature and the frequency shows a steady increase with a rise in temperature (Chadwick, 1953). In the case of triatomid bugs it may be possible to implicate some sort of similar mechanism in the muscles associated with ambulatory locomotion. Whatever the mechanism is, it is clear that temperature influences directly speed of locomotion and hence the total phototactic responses of triatomid bugs.

The influence of ambient relative humidity on the phototactic behaviour of triatomid bugs

The behaviour of arthropods can be influenced dramatically by the ambient relative humidity. The woodlouse, Porcellio scaber (Crustacea), normally found in damp places, e.g. under stones, will vary its general locomotor velocity according to the ambient relative humidity. Thus, in dry air, woodlice are almost incessantly active with very rapid movements, whereas in moist air the average speed of movement is low and the woodlice are often found to stop moving altogether. The end result of this dependence of linear velocity on humidity is that the woodlice tend to aggregate in situations with moist air. This orientation mechanism is described as an orthokinesis and it clearly has a survival value in woodlice which would certainly die if maintained for long periods in dry air.

Most insects have a range of preferred humidities in which they manifest relative inactivity, whereas outside of this range there is a notably increased activity. In general, therefore, insects will tend to avoid a very high humidity, or conversely, a very low one. Schistocerca hoppers are considerably agitated by a relative humidity either side of 60% - 70% (Wigglesworth, 1965). Conversely, adult Tenebrio show a preference for air with a low relative humidity, although individuals subjected to desiccation will exhibit a distinct preference for higher humidities until the internal body water content returns to normal. Clearly the ambient relative humidity is important in the behaviour of some arthropods, their overall biology being geared to a very specific range of relative humidity. The differences obvious in relative humidity preferences may indeed reflect differences in the degree of waterproofing to be found in the cuticle of each insect species.

It is not known from this study how far relative humidity might influence the behaviour of triatomid bugs. The distribution of Triatominae in the wild is undoubtedly influenced by temperature and relative humidity. Lucena (1959) indicates a preferred relative humidity of 80% for both _Panstrongylus megistus_ and _Triatoma infestans_ and 70% for _Triatoma maculata_. These humidities are clearly those to which the species' reproductive capacity and development are adapted and which ensure successful breeding of the species. Furthermore, Gomez-Nunez (1963) states that a humidity of less than 50% might considerably reduce the frequency of eclosion in _Rhodnius prolixus_.

Interestingly, laboratory investigations have revealed that colonies of triatomid bugs are able to withstand a much wider range of relative humidity. Ryckman (1952) states that a range of relative humidity from 32% to 96%, tested at a constant temperature of 30°C., had no serious effect on a laboratory culture of triatomid bugs. Ryckman and Ryckman (1966) have further stated that triatomid bugs are capable of living in a wide range of relative humidities from very high to very low, including a range of 10% - 96%. Clearly, triatomids are hardy insects able to withstand a wide range of relative humidity without any detrimental effect on biological functioning.

The way in which relative humidity might influence the behaviour, and in particular phototactic behaviour, of triatomid bugs has not been thoroughly investigated. A preliminary experiment here suggested that the two relative humidities tested, namely 29% (± 3%) and 90% (± 3%) had no significant effect on the total response of bugs to betalight. Further experiments encompassing a wider range of relative humidities are clearly warranted. Apparatus which allows a precise evaluation of phototactic behaviour in carefully controlled humidities must therefore be designed for this purpose. Nevertheless, the suggestion that relative humidity is not significant in the bugs' response to betalight may indeed be corroborated by other work both on triatomid bugs and other species of insects (Sjogren and Ryckman, 1966; U.S. Department of Agriculture, 1961) which have not indicated a marked relationship between humidity and response to a light source. Wiesinger (1956) has stated that relative humidity is of no importance in determining the periodical activity of Triatoma and that humidity is not in itself a controlling factor in the selection of a suitable resting place. Lumsden (1947) in his study of Aedes aegypti suggests that the environmental relative humidity was of little or no importance in the biting activity of this species of mosquito. Clearly there are arthropods whose immediate behavioural responses are not influenced by ambient relative humidity.

It may be that relative humidity is not important in the short term behavioural responses of bugs. Indeed, no difference in the general activity of bugs, and hence phototactic response, was observed at the range of humidities possible in this study. A relative humidity below 29%, however, might have some effect on activity; indeed, a very low ambient humidity might elicit an orthokinetic reaction, although Wiesinger's study (1956) seems to discount this possibility.

Finally, despite laboratory investigations which suggest a wide ranging tolerance to relative humidity, this environmental factor does appear to have significance, together with temperature, in limiting the distribution of different species of Triatominae in the wild.

This theme clearly warrants further experimentation both in the field and in the laboratory.

The environment of the domestic and peri-domestic habitat and its influence on the operation of light traps

The eventual aim in epidemiological surveys of Chagas's disease should be the extensive use of light trap operations in the domestic and peri-domestic habitat. In that connection a knowledge of the possible seasonal and diurnal fluctuations in climatic conditions within such environments would indeed be invaluable to see how far external environmental conditions affect the catches of bugs in a light trap. There does not appear to have been an extensive study of the climate, and in particular the micro-environment, of human habitations within the endemic zones of Chagas's disease (U.S. Department of Agriculture, 1972; Miles and Rouse, 1970). In the absence of such specific environmental data, the work of Haddow (1942) seems particularly relevant in this context since it provides a thorough description of primitive dwellings in Africa which at this stage may provide a useful comparison. The native huts of Kisumu District in Kenya were generally round buildings, constructed with mud walls and topped with steeply pitched grass roofs. The absence of windows was a particularly notable feature. Haddow recorded the environmental conditions within such a hut by means of thermograph and hygrograph. The results he obtained indicate that a hut will offer a high degree of shelter and protection for the domestic insect fauna such as mosquitoes. The native hut in Kisumu appears to be a remarkably insulated structure in which environmental conditions are equable. The mean ranges in both temperature and relative humidity were considerably less pronounced inside the hut than those of the outside, so much so that Haddow suggests that these two factors might be discounted as factors causing variations in the biting activity of mosquitoes. Haddow clearly demonstrated the differences between indoor and outdoor environmental conditions in cool wet weather and hot dry weather. Thermograph records indicated that differences between indoor and outdoor temperatures were much greater in hot dry weather. Similarly, the differences between indoor and outdoor humidities were much greater in hot dry weather. Temperatures inside the hut during the months October to December ranged from 23°C. at midnight to 26°C. at midday. In wet weather the range was from 20°C. - 26°C., whereas in dry weather it was 21°C. - 31°C. This data at least indicates the kind of daily temperature range inside a habitation and furthermore how this range can be influenced by wet and dry weather. The experimental data in this thesis indicate that bugs are considerably more responsive to betalight at higher temperatures and so it seems reasonable to postulate that the trapping effect might be significantly enhanced in hot dry weather.

In Haddow's study (1942) the relative humidity inside a hut ranged from 58% at midday to 83% at midnight in wet weather. In dry weather the range was 43% to 72%. The experimental findings obtained in this study suggest that relative humidity has no significant effect on the activity of triatomid bugs and so any similar changes in the relative humidity within South American habitations may possibly be disregarded.

Lumsden (1951) carried out a similar investigation of hut environments in Bwamba County, Uganda. Again the housing was primitive, consisting essentially of grass thatch on a framework of poles. The walls of these dwellings were poorly constructed and the absence of windows meant a considerably reduced light intensity inside the huts, being in some cases below that even of the forest environment. Lumsden in his study similarly concluded that the hut provided an environment sheltered from the marked climatic variations of the exterior. Thermograph tracings did not reveal any of the short-term fluctuations which are particularly characteristic of the open air. Although Haddow has suggested a fairly constant environment within the hut with only minimum ranges of temperature and humidity, discounting any effect on mosquito behaviour, there are nevertheless slight variations which, within a similar habitat in South America, would elicit marked differences in bug behaviour. This study has revealed for example that only a few degrees difference in temperature are sufficient to alter the level of phototactic response.

The relevance of the findings in this investigation has yet to be determined. The climatic conditions prevalent in the South American domestic and peri-domestic habitat need to be fully assessed. It may at least be presumed that the environment of both the domestic and peri-domestic habitat will in turn be influenced by the general climate of the immediate area. An intensive climatic survey of the area in which a light trap is to be used is suggested and in this connection, the general review of the climatology of South America by Eidt (1968) in which the author classifies various regions according to meteorological data would seem invaluable.

Chagas's disease occurs principally in rural zones, and furthermore there appears to be a close correlation between the incidence of the disease and the low socio-economic conditions encountered in endemic zones. Rodrigo Zeledon (1974) has underlined in particular the epidemiological significance of sanitary conditions, housing conditions (based on type of construction) and educational standards. In those situations where the housing is primitive, conditions are ideal for triatomid infestation. In general, habitations which are poorly lit, dirty, constructed with mud, adobe or cane walls, and roofed over with thatch, will provide shelter for species of Triatoma and Panstrongylus within the cracks and fissures of the walls. (There is some similarity with the African huts described by Haddow (1942) and it may be supposed that South American dwellings also provide fairly equable environments with similar patterns of temperature fluctuation). Even houses constructed with brick and roofed over with corrugated iron, will, if badly-built, provide harbourage for bugs. Furthermore, the bedding of both humans and domesticated animals will also furnish an excellent resting place for bugs (Usinger, 1944). Bugs have even been located behind old picture frames. Interestingly, species of Rhodnius show a preference for roofs thatched with palm leaves or grass. (Zeledon, 1974).

The environmental conditions within any such habitation will depend therefore not only on external climatic conditions but also, to some extent, on the kinds of material used in its construction. The presence of domestic stock may also be relevant in determining environmental conditions. The total number of human occupants will also affect climatic conditions within any habitation for as Haddow discovered (1942), a hut occupied by ten men has a slightly higher temperature than a hut occupied by only one man. Furthermore the more crowded hut had a somewhat lower relative humidity than the hut occupied by only one man. Clearly, a whole range of factors will be important in creating the overall climate of any human dwelling which will in turn influence the behaviour of any vector species present.

Climate has been designated as a major factor affecting the importance of the epidemiologically significant vector species, and hence both the distribution and rate of transmission of Chagas's disease in South America (Rodrigo Zeledon, 1974). It is suggested, for example, that *Panstrongylus megistus* may not become adapted to human dwellings in some regions simply because the ambient relative humidity is not adequate for continued breeding of the species. A relative humidity below 60% (at a temperature of 21°C. - 28°C.) is regarded as deleterious. There are interesting differences in the overall climatic preferences of triatomids, *Triatoma infestans*, for example, preferring a warm yet at the same time not too humid environment, whereas other species will not tolerate a relative humidity below 60%.

Furthermore, in terms of general seasonal changes, temperature is important in influencing reproduction and development of the insect (Symes et. al., 1962). The distribution of bugs according to temperature has been outlined by Bustamente (1957) and Lucena (1959). *Triatoma maculata* appears to be limited by a range of 23°C. - 27°C. and *Triatoma infestans* by a range of 17°C. - 23°C. Gomez-Nunez (1963) indicates that *Rhodnius prolixus* will tolerate a range of 16°C. - 34°C. These are the preferred temperature ranges of each species, but it must be remembered that, within their respective habitats, there will be considerable daily fluctuations either side of these values. Laboratory experiments carried out by the author and Wiesinger (1956) indicate that temperatures below 20°C. will inhibit activity. It is therefore suggested that should temperatures within habitations drop below 21°C. during the night, there might be a corresponding drop in the size of the catch taken in a light trap. A progressive increase in temperature above 21°C. will yield a progressively bigger catch. Laboratory experiments have indicated that in the case of triatomid bugs, there is something like maximum activity at around 36°C. If night time temperatures reached this level within South American dwellings then, provided the bugs were of the right physiological condition, it might be supposed that the trapping effect would be maximal. Interestingly, Lumsden (1947) discovered that the optimum environmental temperature for biting activity in Aedes aegypti was approximately 35°C.

As far as South American habitations are concerned it may be supposed that a temperature range of 20°C. - 36°C. should provide a reasonable catch of bugs, with a larger proportion of bugs being caught in the upper part of this range. Haddow's study (1942) indicated that temperatures were a few degrees higher in the hours just prior to midnight. It may be that as soon as it is dark, bugs will become active and emerge from crevices while temperatures are still above the fairly low midnight value, e.g. 21° - 23°C. is the temperature recorded at midnight in the African hut (at least from October to December). It could be that in South American habitations all activity, and hence the possibility of a response to a light trap, will be concentrated in the several hours of darkness preceding midnight.

Considerably more investigation is clearly warranted inside human dwellings in South America with particular regard to temperature and its influence on the operation of a suitably designed light trap.

The combination of betalight and butyric acid as an attractant for triatomid bugs

There are various references in the literature to situations where the use of chemical attractants in light traps has considerably increased the size of the insect catch. These have been discussed in some detail in the general review on light traps.

It is significant that triatomid bugs have been shown to exhibit a distinct attraction to the saturated fatty acid, butyric acid ($CH_3CH_2CH_2COOH$, b.p. 162°C.). Butyric acid, like the other lower saturated fatty acids, has a decidedly unpleasant and penetrating smell. Indeed, the odours of perspiration and rancid butter are chiefly due to the presence of butyric acid and it is further interesting to note that the acid has been shown to occur in the free state in human sweat (Hey, 1966). Since bugs may be attracted to their hosts by various chemical emanations, e.g. expired carbon dioxide and the odour of sweat, it seemed reasonable to infer that bugs might be attracted to butyric acid, a constituent of human sweat. Interestingly, fourth instar nymphs of *Rhodnius prolixus* manifested a diel rhythm of attraction to a source of butyric acid. The finding is significant in that there is at least available a ready supply of synthetic chemical attractant which could play an important role in the trapping of triatomid bugs in the field. Beroza (1970) gives an interesting account of the possible use of synthetic chemical attractants in controlling some of the economically important pests. The same theoretical considerations outlined by Beroza (1970) may equally apply to the medically important insect vectors.

This investigation has been concerned with the possibility of incorporating a successful synthetic chemical attractant in a light trap suitable for triatomid bugs. In this connection it may be further useful to experiment also with caproic acid, $C_5H_{11}COOH$, the skin secretion of goats, and acetic acid, CH_3COOH, which has already been shown to be attractive to *Glossina* (Chapman, 1961). These may also prove to be useful attractants for triatomid bugs. In this study, catches of third instar nymphs of *Triatoma infestans* were found to be considerably larger in a choice chamber equipped with a combined attractant, namely betalight and n-butyric acid. This finding may have relevance in field conditions with other stages and species.

The application of chemical attractants for trapping bugs in the field has not been fully investigated as yet and some experimentation on this theme is certainly warranted. There may even be some possibility of using synthetic pheromones in light traps set to catch triatomid bugs. This technique has proved considerably successful in work on other insect groups, most notably that of Henneberry et. al. (1967) which indicated that the light trap catches of male cabbage looper moths, *Trichoplusia ni* could be considerably increased by baiting the traps with virgin females. The work of Howland et. al. (1969) has shown that light traps also equipped with synthetic pheromone significantly increased the catches of males of *T. ni* over a period of several weeks. In this connection it is may equally apply to the medically important insect vectors.

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In conclusion, it is important to stress that it is not sufficient to simply observe attraction to a chemical source and assume that this will prove a successful attractant in the field. The entomologist has to design a trap which will yield a maximum catch; he has to check the size and type of chemical dispenser and also the concentration of chemical, remembering that it may repel the insect at high concentrations; finally, the entomologist will need to consider not only the size of the trap openings but also the actual trapping technique, for example, whether a volatile insecticide will be utilized, a sticky substance, or simply a mechanical baffle. Whether the attractant works in field conditions depends not only on some of the factors mentioned in the review of literature on light traps but also the direct competition from other ambient stimuli which could conceivably override the influence of the attractant.

CONCLUDING REMARKS

The attraction of an insect to light will be influenced by many factors. These factors, both environmental and physiological, have been outlined by Weiss et.al. (1943) and a diagram indicating the possible interaction between these factors is presented in Figure 35. These same factors can of course influence the attraction of vector species, and in particular triatomid bugs, to a light source. The relative significance of each factor mentioned by Weiss et. al. (1942) is discussed in the context of experimental findings relating to triatomid bugs.

The sensory apparatus, receiving the light stimulus, will be subject to some variation in functional operation. The way in which the compound eyes and ocelli respond to the light source may depend on a number of factors:

1. The insect's previous exposure to light, in other words, whether it is light adapted.
2. The angle of incidence
3. Variations in sensitivity in the different regions of the compound eye
4. Position of the iris pigment
5. Rate of pigment migration in relation to light intensity.

The question of sensory adaptation was not studied experimentally here in this study but it is suggested that this may indeed be important where a considerably brighter light source than betalight is used in field operations. Furthermore, the internal structure of the compound eyes and ocelli of triatomid bugs will need to be investigated fully to build up a complete understanding of phototaxis in triatomid

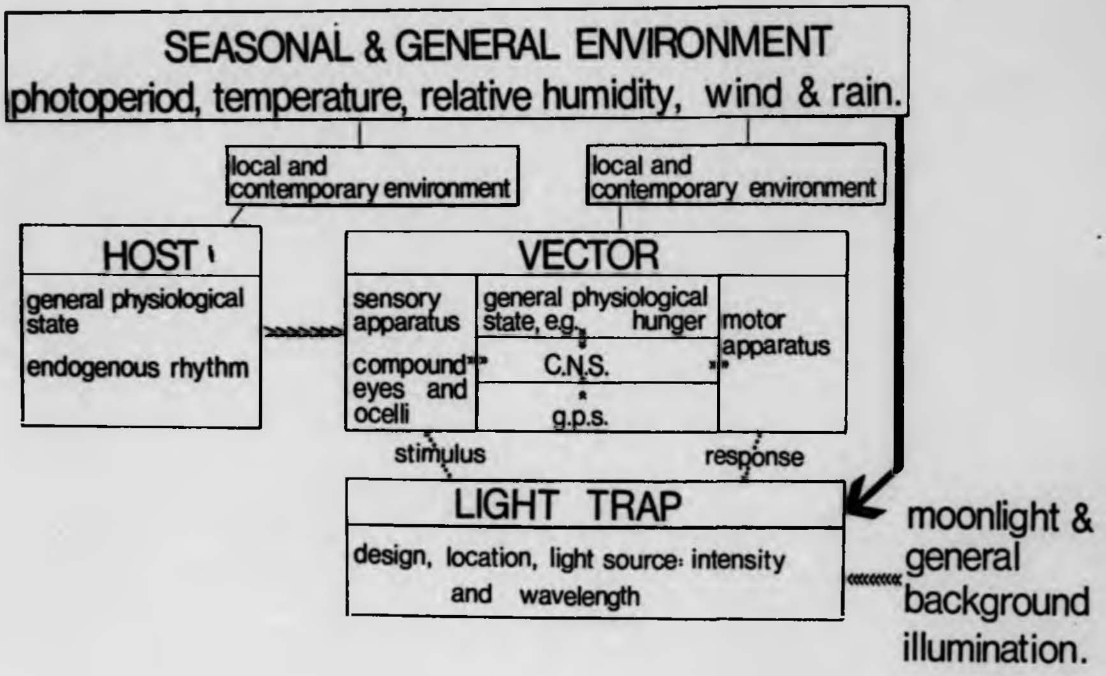

Figure 35. Interacting factors likely to influence the attraction of triatomid bugs to a betalight trap.

bugs.

Provided the sensory receptors are ready physiologically to receive the light stimulus and transmit this information in the form of nerve impulses to the brain, then the insect will respond and behave accordingly. However, the way in which the vector responds to a light stimulus, emitted by a light trap, will ultimately depend on its physiological condition. Observations from experimental work in this thesis suggest the importance of the nutritional condition of bugs, bugs manifesting a gradually enhanced phototactic response with progressive starvation. Furthermore, the ecdysis or moult has been shown to considerably depress the phototactic response. The week or so prior to the moult will see a gradual decline in phototactic behaviour and it is suggested that this may reflect the level of metabolic reserves available for locomotor activity. The ways in which bugs respond to a light source post-emergence has indicated that, in the absence of a blood meal, there is a gradually increased response to light. This may again reflect the use or state of the insect's metabolic reserves. Finally, the absence or presence of a suitable host will clearly influence the physiological state of a triatomid bug and so this factor must be included in the consideration.

The operative efficiency of a light trap will show a good deal of variability. The brightness of a light source is perhaps foremost as a factor likely to cause such variability. Triatomid bugs manifested a significantly greater response to betalights of higher microlambert value. The influence of colour needs to be investigated further but experimental work here suggests, perhaps tentatively, that the shorter wavelengths of radiation might prove considerably more attractive to triatomid bugs. The overall design of a light trap also needs careful consideration such that the finally formulated trap is adequate to catch a sufficiently large enough collection of specimens, or, at least, a number or sample of insects suitable to the needs of the investigator. Having achieved an optimum trap on the basis of both general design and light source, it is then important to ensure that the trap is sited in the appropriate location so that it may operate at its maximum efficiency.

An insect's physiology will be very much influenced by its local and contemporary environment. Weiss et. al. (1943) have suggested temperature, relative humidity, air currents, and ambient chemical stimuli as possible factors which may affect the way in which an insect responds to light. Experimental design precluded a full investigation of relative humidity and its influence on the phototaxis of triatomid bugs. However, on the basis of limited data, the author suggests that even though ambient relative humidity is known to influence the ecological distribution of a bug, and, in the long run, its adaptive and breeding success, there is no reason to suppose that this environmental factor might influence a bug's more short-term behavioural response to a light source. Temperature, however, has been shown to have a very profound influence on bugs' phototactic responses. A considerably enhanced response to a betalight source results at more elevated temperatures and this has been shown to depend on the direct effect of temperature on speed of movement. A temperature of around 36°C. will certainly yield something like a maximum response whereas temperatures below 21.7°C. will, as suggested by experimental data presented here, considerably reduce the bug's potential of responding to a light source. Temperature may ultimately affect the bug's general level of metabolism. It would indeed be interesting to see how close the correlation might be between light trap catch and ambient temperature in the field. An extensive study of climatology and its relation to trapping is to be strongly recommended.

The vector will also be subjected to a range of ambient chemical stimuli. Emanations from the host in the form of carbon dioxide and sweat will be foremost in enabling the bug to locate its blood meal. It is therefore significant that butyric acid has proved attractive to bugs and it has been suggested that this chemical might be used in conjunction with a light source as a combined attractant in a suitable trap. This is clearly an example of a synthetic chemical being used to enforce or strengthen the trapping effect achieved with a light source. Conversely, it is important to stress the possibility of extraneous chemical stimuli overriding the trapping effect of a photostimulus in situations where only a light source is employed in a trap. Air currents, although not considered in this investigation, are of course important dispersal agents for chemical stimuli.

It is not known how photoperiod might affect the behaviour of bugs in the domestic and peri-domestic habitat. Laboratory investigations have, however, indicated that a bug's previous photoperiodic entrainment has the capacity to determine the onset and duration of its locomotor activity.

A pre-arranged set of time cues or zeitgebers in the form of definite cycles of light and dark will govern, very precisely, the bug's behaviour such that, in the absence of further zeitgebers, a diel pattern of locomotor activity is manifested over a period of several days. Experiments have clearly shown that triatomid bugs only manifest maximum activity during the period corresponding to the scotophase or dark cycle of their photoperiodic entrainment. Furthermore, the phototactic responses of bugs have been shown to follow closely the diel rhythm of locomotor activity as governed by photoperiodic entrainment.

Bugs, adapted to the nocturnal habit, emerge from their crevices when it is sufficiently dark. Interestingly, laboratory investigations by Wiesinger (1956) have clearly shown an inhibition of feeding activity in Triatoma infestans in the presence of strong light. Furthermore, it is suggested that the choice of a hiding place some time after a blood meal may be governed by a combination of photophobia and thigmotaxis. Light and darkness are therefore important in governing the periodical activity of bugs in the wild. It seems reasonable to postulate that the natural photoperiod will also initiate distinct rhythms of behaviour, both in terms of locomotion and feeding.

In a consideration of the possible mechanisms underlying the attraction of an insect to a light source it was stated that the amount of contrast existing between the light source and its immediate surroundings will be of primary importance in determining the extent to which a light trap may catch insects. Moonlight, or indeed any other general background illumination will reduce this level of contrast and therefore interfere with the insect's phototactic response. Background illumination will therefore have a considerable effect on the operative efficiency of any light trap and should also be considered seriously in any epidemiological survey which aims to use light traps as a means of collecting triatomid bugs. Some knowledge of the general levels of illumination in dwellings, both by day and by night, will be particularly valuable in this context. Many of the poorly constructed dwellings in South America are fairly dark even during the day which suggests that, in the absence of any artificial illumination at night, a light trap should operate reasonably well.

This investigation has been concerned primarily with the phototactic responses of triatomid bugs, and more specifically, their attraction to the self-powered light source called betalight. A positive diel rhythm of attraction to a wide range of betalights, of different brightnesses and different colours, has been demonstrated not only in the five species studied here but also in their different instars. The results from this preliminary laboratory investigation therefore suggest that betalight, with all its inherent advantages, could prove a useful attractant for a light trap in the field. The next step in this study should be to assess the possibility of incorporating betalight in a trap of suitable design, and this will necessitate extensive experimentation in the field. The experience gained in this study therefore constitutes a useful theoretical background for such experimentation and indeed Figure 35 should offer a useful guide line for the entomologist engaged in any epidemiological survey.

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