Effects of temperature, moisture and thermal acclimation on the biology of Tenebrio molitor (Coleoptera: Tenebrionidae)

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Effects of temperature, moisture and thermal acclimation on the biology of *Tenebrio molitor* (Coleoptera : Tenebrionidae)

by

Fred Punzo

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

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INTRODUCTION

In the present study, several aspects of the temperature relationships of the mealworm or darkling beetle, Tenebrio molitor (Coleoptera: Tenebrionidae) were investigated. Although not many members of this family are of economic importance, the genera Tribolium (flour beetles) and Tenebrio do contain several pest species of stored grain products (Cotton, 1927). Species of the genus Tenebrio are dark in color and are generally found in decaying vegetation where moisture conditions are high; most species feed on fungi (Scholz, 1925). In its ecology, however, T. molitor differs considerably from all but a few of the members of this genus in that it is an introduced species (Cotton and George, 1929) which frequents stored grain bulks (Howard, 1955). The larvae are elateriform, yellow in color and less sclerotized than the adults. Both the larvae and adults are detrimental to stored grains although neither is destructive to living crops (Arendsen, 1920; Dillon and Dillon, 1961).

Although T. molitor has been used rather extensively as an experimental animal (Halstead, 1963; Patton and Craig, 1939; Socha and Sehnal, 1972; Tracey, 1958; Urs and Hopkins, 1973; Yinon, 1970), many aspects of its biology are not clearly understood. As was previously mentioned, the earlier investigations on the effects of temperature and humidity on this species (Buxton, 1930; Dodds and Ewer, 1952; Gunn and Pielou,
were performed in the absence of stringent humidity controls.

There has been an emphasis in recent years on studies concerning the biology and behavior of stored product insects and the effects of certain environmental factors on their survival capacity. Areas which have received the most attention with respect to temperature relations have included studies on temperature preferences in the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Amos, 1968, 1969), the flour beetles of the genus *Tribolium* (Amos and Waterhouse, 1969; Graham, 1958; Gray, 1948; Roth and Willis, 1950; Waterhouse et al., 1971; Willis and Roth, 1950), and the mealworm *T. molitor* (Mellanby, 1932); studies on the effects of temperature and humidity on survival capacity in the grain weevil, *Sitophilus granarius* (Bailey, 1969; Howe and Hole, 1968; Robinson, 1926), the ptinid beetle, *Ptinus tectus* (Bentley, 1944), the flour beetles, *Tribolium confusum* and *T. castaneum* (Howe, 1956, 1960; Watters, 1966), the book louse, *Liposcelis* sp. (Knulle and Spadafora, 1969), the cigarette beetle, *Lasioderma serricorne* (Lefkovitch and Currie, 1967) and the cereal leaf beetle, *Ovlema melanopus* (Wellso, 1973); investigations on the relationship between temperature and distribution in *T. castaneum* and *T. confusum* (Amos and Waterhouse, 1969; Cotton, 1950; Jay et al., 1971), in the rice weevil, *Sitophilus*
oryza (Robinson, 1926) and the khapra beetle, Trogoderma granarium (Yinon and Shulov, 1969); studies on the effects of temperature on development in T. confusum and T. castaneum (Howe, 1956, 1960; Park and Frank, 1948; Watters, 1966), S. granarius (Howe and Hole, 1968) and O. melanopus (Wellso, 1973); and the effects of temperature on fecundity in T. confusum (Howe, 1960), T. castaneum (Park and Frank, 1948), L. serricorne (Lefkovitch and Currie, 1967), and S. granarius (Surtees, 1964a). There have also been several studies concerning the relationship between temperature and moisture on locomotor activity in S. granarius (Barlow and Perttunen, 1966; Barlow and Kerr, 1969; Howe, 1951) and T. confusum (Howe, 1960; Surtees, 1964a) and dispersion behavior in Lepinotus partruelis (Fahy, 1971), T. castaneum (Hagstrum, 1973; Proctor, 1972), S. granarius (Surtees, 1963), Crypoto lestes ferrugineus (Surtees, 1964b), O. surinamensis (Surtees, 1964b, 1964c, 1964d), and T. molitor (Ernst and Mutchmor, 1969). However, only one of these studies analyzed dispersion in a three-dimensional gradient (Surtees, 1964b); the other studies were two-dimensional in scope. In addition, the effects of temperature and moisture on the physical characteristics of the grain itself which are vital to the survivorship of stored product species, have been investigated by several workers (Denmead and Bailey, 1966; Gay, 1946; Howe, 1962; Pixton, 1967, 1970; Pixton and Griffiths, 1971; Pixton and Warburton, 1968, 1971).
The purpose of the present study was to ascertain the effects of temperature, moisture and thermal acclimation on *T. molitor* with respect to the following parameters:

1. survival capacity of the various developmental stages of this insect
2. changes in temperature tolerance during larval development
3. the egg-laying rates of adult females
4. the longevity of the adults
5. locomotor activity of larvae and adults
6. three-dimensional dispersion behavior of larvae and adults.
LITERATURE REVIEW

General

Terrestrial existence poses a number of problems for arthropods which are greatly accentuated in hot-dry environments. Although the expression, "hot-dry environment", is most often associated with desert conditions, a comparable set of physical conditions is frequently encountered within the microhabitats of stored grain environments (Cloudsley-Thompson, 1970). The basic problem confronting terrestrial life-forms, especially in xeric habitats, is one of preventing evaporative water loss. Insects and other arthropods are characterized by a very large surface area to volume ratio and, as a result, may transpire water readily. It is for this reason that the conservation of water is of paramount physiological importance.

Many arthropods such as millipedes, centipedes and collembolans, resist the desiccating properties of the atmosphere by remaining within the moist confines of the soil. These arthropods generally lack the waxy epicuticular layer associated with the integument of insects, arachnids and mites (Arlian and Wharton, 1974; Blower, 1951; Bursell, 1955; Davies, 1928; Davies and Edney, 1952; Edney, 1967; Holdgate and Seal, 1956; Lees, 1947) which functions as an effective barrier to water loss through the integument (Beament, 1954; Eder, 1940; Edney, 1954a, Johnson, 1942; Manton and Ramsay, 1957; Richards, 1951; Thornwaite, 1940; Waloff, 1941; Wigglesworth, 1957).
However, such a mechanism for water resistance also makes the cuticle of these animals relatively impervious to the diffusion of oxygen and carbon dioxide as well (Chapman, 1971; Wigglesworth, 1965). This severely restricts the degree to which cuticular diffusion can meet the respiratory demands of the animal. As a result, insects and other arthropods have evolved respiratory systems which facilitate gaseous exchange while simultaneously restricting evaporative water loss (Allee et al., 1949; Cloudsley-Thompson, 1962a; Herter, 1953; Lindauer, 1954a; Uvarov, 1931). For example, the book lungs of arachnids and the spiracles of insects are provided with muscles which function in the opening and closing of these systems (Vollmer and MacMahon, 1974; Wigglesworth, 1965). It has been demonstrated that when the carbon dioxide levels in the tissues increase to a certain level, these muscles are caused to relax, thereby opening the respiratory system and facilitating the removal of carbon dioxide and the intake of oxygen (Bursell, 1964a; Chapman, 1971; Wallwork, 1960; Wigglesworth, 1957). In addition to the evolution of specialized respiratory structures, arthropods exhibit other adaptations for water conservation. Prior to molting, a new epicuticular waxy layer is secreted before the old cuticle is shed, thus ensuring minimal water loss during ecdysis (Edney, 1957; Lees, 1947; Wigglesworth, 1965). Also, insects and arachnids have evolved metabolic excretory products in the form
of highly insoluble uric acid and guanine. In this way nitrogenous wastes can be eliminated with minimal water loss (Browning, 1934; Davies and Edney, 1952; Dresel and Moyle, 1950; Edney, 1967; Maloeuf, 1938; Schmidt-Nielsen, 1964).

A number of authors (Allee et al., 1949; Cloudsley-Thompson, 1962a, 1970; Fraenkel and Herford, 1940; Kirchner, 1973; Lindauer, 1954b) have arbitrarily divided arthropods on an ecological basis into two general classes. The first includes those forms which do not possess a highly differentiated epicuticular waxy layer and thus lose water rapidly by transpiration through the integument. Most of these forms are characterized by behavioral mechanisms which ensure a positive orthokinesis in a gradient of humidity (Bursell, 1957; Buxton, 1924b; Fraenkel and Gunn, 1940; Heinrich, 1973; Meyer and Raffensperger, 1974; Sioli, 1937) and cycles of nocturnal activity (Cloudsley-Thompson, 1959, 1961; Marler and Hamilton, 1966). The second group includes arachnids and insects which exhibit a higher degree of resistance to evaporative water loss due to the highly differentiated waxy layer of the epicuticle which characterizes these species.

Because water comprises a high percentage of the tissues of arthropods and the basic problem of terrestrial existence is the conservation of this water, environmental humidity and temperature are of vital importance to the biology and survivorship of this group.
Although the effects of temperature have been studied more extensively than any other environmental factor with respect to the biology of arthropods (Allee et al., 1949; Bursell, 1964a; Chapman, 1971; Cloudsley-Thompson, 1970; Uvarov, 1931; Wigglesworth, 1965), a great deal of work remains to be done.

Temperature not only plays an important role as a limiting factor in the geographical distribution of animals but also exerts a critical influence on their rate of activity (Kerkut and Taylor, 1958; Richards, 1956). Those organisms which possess body temperatures that closely parallel those of their environment are often referred to as poikilotherms while those which possess a higher degree of thermoregulatory control are referred to as homeotherms (Precht, 1968; Prosser, 1961). It has been argued, however, that the term poikilothermic implies an absence of thermoregulation and it is a well-known fact that some degree of thermal control, whether behavioral or physiological, is manifested by arthropods in general (Clench, 1966; Dainton, 1954; Heinrich, 1973; Kevin and Shorthouse, 1970). In view of this fact, Cowles (1962) introduced the term ectothermic as preferable to poikilothermic because it implies that, although the body temperature is derived largely from the environment, there is some degree of behavioral or physiological control. Behavioral thermoregulation by terrestrial invertebrates is accomplished either by various orientation movements towards a heat source or by the active avoidance of
temperature extremes through burrowing, aggregation or by the evolution of definitive diurnal or nocturnal activity cycles.

Behavioral Thermoregulation

Perhaps the most vital behavioral adaptation to environments characterized by extremes of temperature is the development of burrowing habits (Buxton, 1924b; Cloudsley-Thompson, 1961; Schmidt-Nielsen, 1964). This is most readily apparent in annelids, mollusks and arthropods as well as in many species of xeric rodents. Annelids will burrow deeply into cooler and wetter regions of the soil during the summer months and thus maintain themselves under more optimal conditions (Cloudsley-Thompson, 1964; Flemister, 1964). Fossoriality is widely developed in the Arthropoda. The centipede fauna of xeric areas consists principally of burrowing forms (Aschoff, 1963; Cloudsley-Thompson, 1959; Seymour, 1974). Many insects, especially tenebrionid beetles, have morphologically flattened bodies which facilitate burrowing into the soil or beneath the surface of loose sand (Cloudsley-Thompson, 1964; Edney, 1967; Wigglesworth, 1965).

Another obvious behavioral mechanism of thermoregulation utilized by terrestrial invertebrates is the orientation of the body axis in such a way as to increase or decrease absorption of radiant energy (Fraenkel and Gunn, 1940; Kennedy, 1937). Most arthropods actively avoid excessively high or low
temperatures by a klinokinetic movement away from a source of heat (Fraenkel and Gunn, 1940). In general, a great many invertebrates respond to the physical parameters of the environment through bodily movements that are proportional to the intensity of stimulation (Heinrich, 1973; Meyer and Raffensperger, 1974; Miller, 1929; Nieschulz, 1935; Parry, 1951; Pepper and Hastings, 1952; Pienkowski and Golik, 1969; Sweetman, 1939). Several tropical termite species ensure favorable nesting temperatures by orienting their bodies in a north-south direction, thereby minimizing the area exposed to the noonday sun (Cloudsley-Thompson, 1964; Luscher, 1961; Wilson, 1966). This provides an example of a two-fold behavioral thermoregulatory mechanism in that a specific orientation is utilized in addition to the fossorial habit of the species. Larvae of the Nearctic ant lion, Myrmeleon immaculatus move themselves within their burrows in such a way as to maintain a position which places them in the area of optimal temperature (Cloudsley-Thompson, 1971). During the lower temperatures of early morning they are found on the south and east areas of their pits whereas in the afternoon they position themselves to the south and west. In lower ambient temperatures, several species of locusts orient themselves so that the axis of their body is at right angles to the direction of the sun's rays thereby exposing the maximal body surface area to radiant heat and thus achieving some degree of
thermoregulation (Clarke, 1960; Digby, 1955; Fraenkel and Gunn, 1961; Hall and Root, 1930). As soon as the ambient temperature rises above that preferred by the species in question, the locusts reorient themselves by directly facing the sun; this reduces the area of the body exposed, thereby effectively reducing body temperature. This basking activity is an example of a directed response toward radiant energy. The North American blood-sucking bug, *Rhodnius prolixus*, will orient its body toward a source of heat even after the eyes have been surgically removed (Wigglesworth and Gillett, 1934). Larvae of *Porthesia chrysorrhoea* will direct the lateral aspects of their body toward the sun when ambient temperatures are below 23°C (Fraenkel and Gunn, 1961). The bedbug, *Cimex lectularius*, also exhibits a positive klinotaxis towards a source of heat (Sioli, 1937). The literature concerning orientation movements by arthropods is rather extensive and is reviewed in detail by several authors (Fraenkel and Gunn, 1940, 1961; Marler and Hamilton, 1966).

The effects of temperature are often considerably modified by existing humidity conditions, and frequently orienting mechanisms are directed toward humidity gradients. For example, the woodlouse, *Porcellio scaber*, exhibits a definite hygro-kinesis. This animal is normally found in loose soil and leaf litter and cannot tolerate prolonged dry periods. In an
experimental chamber which provides a gradient in relative humidity from dry to moist conditions, *P. scaber* will orient and move toward the more humid areas in the gradient (Edney, 1954a, 1957; Gunn and Kennedy, 1936; Kennedy, 1937). Several insect and arachnid species are negatively hygrotactic in moist air (Heinrich, 1973; Kirchner, 1973) but this response is often reversed under arid conditions (Cloudsley-Thompson, 1962a, 1964; Kirchner, 1973). For example, the humidity behavior of *Tribolium confusum* and *T. castaneum* gradually changes with progressive conditions of aridity from an initial preference for the lower of two humidities to a preference for moist conditions (Amos and Waterhouse, 1969; Fraenkel and Gunn, 1940; Gray, 1948; Howe, 1956; Park and Frank, 1948). This has also been demonstrated for woodlice (Edney, 1947, 1957, 1967), several species of tenebrionid beetles (Cloudsley-Thompson, 1962a, 1964; Deal, 1941), arachnids (Almquist, 1970; Madge, 1961; Kirchner, 1973) and millipedes (Cloudsley-Thompson, 1971; Gunn, 1942).

Another important behavioral mechanism which affords some degree of thermoregulation is the evolution of definitive nocturnal patterns of activity which ensure the presence of favorable environmental conditions when the species is active. The adaptive significance of such circadian rhythms is apparent and is discussed in detail in several reviews (Aschoff, 1963; Brown, 1959; Bruce, 1960; Chapman, 1971; Cloudsley-Thompson,
1961; Harker, 1964; Marler and Hamilton, 1966). Various behaviors such as feeding, locomotor activity, vocalizations and others are examples of cyclic behavior patterns. In the case of terrestrial arthropods, nocturnal habits are intimately associated with the need to conserve water, especially in xeric species. Those arthropods which remain diurnal in their activity frequently possess relatively long appendages which effectively raise them off the surface of the ground. In addition, the legs are frequently utilized in stilting movements which increases the distance between the body of the animal and the substrate where temperatures frequently exceed the viable tolerances of the species in question (Hamilton, 1971).

Physiological Thermoregulation

In addition to behavioral mechanisms of thermoregulation, there are several physiological mechanisms of temperature control exhibited by terrestrial invertebrates. These include slime secretions by annelids and mollusks (Bursell, 1964b; Cloudsley-Thompson, 1959, 1964), and the utilization of metabolic heat, the absorption of radiant heat and evaporative cooling by transpiration through the cuticle utilized by arthropods (Bursell, 1964a; Cloudsley-Thompson, 1970; Heinrich, 1973; Eder, 1940; Edney, 1954b, 1957, 1967; Flemister, 1964; Precht, 1968; Prosser, 1955; Wigglesworth, 1965).
The evaporative loss of water through the integument results in a cooling effect (lowering of body temperature) because the latent heat of vaporization is withdrawn from the body (Clarke, 1967; Edney, 1957; Mellanby, 1954a). In arthropods that possess a relatively thick epicuticular waxy layer which is highly impermeable to water, evaporative water loss is negligible. However, many species of millipedes, centipedes, arachnids and insects are capable of regulating their body temperature through evaporative cooling for short periods of time (Cloudsley-Thompson, 1962b; Hanec and Beck, 1960; Heinrich, 1973; Mellanby, 1932; Uvarov, 1931; Wigglesworth, 1965). For this reason they are able to withstand higher temperatures in an arid environment as compared to one of high relative humidity (Edney, 1967). In a dry environment evaporation occurs rapidly, and this can result in the body temperature of an insect being 3° - 4°C below the ambient temperature (Cloudsley-Thompson, 1970; Wigglesworth, 1965). The rate of evaporation is also affected by the ambient temperature. Generally, the rate of evaporation is lower at low air temperatures, so that the body temperature of an arthropod slightly exceeds the air temperature due to metabolic heat gain (Prosser, 1955, 1961).

At higher temperatures the rate of evaporation is increased, thereby causing the body temperature to fall below the ambient temperature (Clarke, 1967; Wieser, 1973). These
modifying influences of temperature on the rate of transpiration through the cuticle are due to the fact that temperature not only affects the drying power of the air but also the permeability of the cuticle itself (Arlian and Wharton, 1974; Beament, 1954; Blower, 1951; Bursell, 1955; Eder, 1940; Edney, 1957, 1967; Edney and Spencer, 1955; Lees, 1947; Treherne, 1954; Uvarov, 1931; Wigglesworth, 1945, 1965).

The extent to which an insect can effectively utilize evaporative cooling as a mechanism of thermoregulation depends on the size of the animal. Smaller insects have an increased surface area to volume ratio and as a result could only lower their body temperature by evaporating more water than they can tolerate losing (Cloudsley-Thompson, 1970; Mellanby, 1954a). In this case, mortality can be attributed to desiccation.

In addition to the mechanisms available for minimizing evaporative water loss across the integument, it has been shown that some arthropod species are able to absorb moisture from unsaturated air (Breitenbrecher, 1918; Cloudsley-Thompson, 1970). This has been experimentally verified in the larvae of *Tenebrio molitor* (Buxton, 1930; Cloudsley-Thompson, 1964; Mellanby, 1932, 1958). If these larvae are maintained at a relative humidity of 80% or lower at 23°C they will gradually lose weight. However, they will gain weight if kept at 90% humidity. This weight gain reflects an increase in the water content of the body. Govaerts and Leclerq (1946) exposed
T. molitor larvae and adults to saturated air containing 8% heavy water. Samples of water extracted from the insect tissues after several hours were shown to contain 8% heavy water indicating that some type of equilibrium between air moisture and tissue water content had been attained. The absorption of water from air has also been demonstrated in the flea, Xenopsylla brasiliensis, even at humidities as low as 50% regardless of temperature (Edney, 1947). Similar capacities have been shown for ticks (Browning, 1934; Lees, 1947; Wigglesworth, 1965). This type of absorption of water from the air can occur either through the cuticle of arachnids or through the tracheal system of insects during respiration (Andrewartha and Birch, 1954; Arlian and Wharton, 1974; Beament, 1954; Blumberg, 1971; Burkett, 1962; Bursell, 1955; Edney, 1957; Edney and Spencer, 1955; Murray, 1968; Ramsay, 1964).

Another physiological mechanism which imparts some degree of thermoregulation to ectotherms is the utilization of metabolic heat (Chapman, 1971; Clarke, 1967; Cloudsley-Thompson, 1964, 1970, 1971; Heinrich, 1973; Wieser, 1973; Wigglesworth, 1965). Metabolic heat gain may be considerable in arthropods and the heat produced by metabolic activities increases the body temperature so that individuals will tend to be slightly warmer than the environment. This is especially true at high humidities where the effects of evaporative cooling are reduced.
In insects, the metabolic heat produced by the thoracic musculature during flight is particularly important in this respect. Thoracic body temperatures in excess of 20°C over ambient temperatures have been recorded for several species of Coleoptera, Hymenoptera and Lepidoptera (Church, 1960; Clarke, 1960; Cloudsley-Thompson, 1964; Pringle, 1965). The thoracic body temperature of the butterfly, Vanessa cardui, is raised from 35°C while perching, to 37°C when in flight (Pringle, 1965). The body temperature of the saturniid moth, Samia cecropia, is raised 4°C during flight (Cloudsley-Thompson, 1970). This mechanism of metabolic heat gain is an important one in that the flight muscles of most insects can only function efficiently within a limited range of temperatures. The sphinx moth, Celeria lineata, maintains a mean thoracic temperature of 38°C by vibrations of the wings even at ambient temperatures as low as 16°C (Adams and Heath, 1964). Although it is capable of flight at thoracic temperatures of 25° - 27°C, this preflight warm-up activity ensures that the insect will fly at optimal efficiency. The silk moth, Rothschildia jacobae, regulates its thoracic temperature during flight within the range of 32° - 36°C at ambient temperatures of 17° - 29°C (Parry, 1951). Heat loss from large moths and other insects which results from evaporative cooling is counteracted by increasing general muscular activities which result in an increased rate of metabolism (Prosser, 1961; Wigglesworth, 1965). It has been argued by several authors (Cloudsley-
Thompson, 1962a, 1970; Marzusch, 1952b; Precht, 1958) that during such periods of increased metabolic activity these insects are actually endothermal. However, they differ from birds and mammals in that the duration of the regulatory period and the range of variation in body temperature that can be tolerated are significantly less (Prosser, 1955, 1961).

The utilization of metabolic heat as a means of temperature regulation is commonly exploited by numerous species of social insects (Allee et al., 1949; Cloudsley-Thompson, 1964; Gates, 1914; Himmer, 1932; Michener, 1969; Uvarov, 1931; Wilson, 1971). The ability to regulate the temperature of nest environments is characteristic of the social insects as a group. Thermal control is accomplished through the architecture of the nest itself as well as through behavioral responses of individual colony members (Lindauer, 1954b). During winter months, many of the colonial Hymenoptera aggregate, thereby raising the internal temperature of the nest considerably above the outside temperature (Cloudsley-Thompson, 1970; Gates, 1914). Metabolic heat is used to increase nest temperatures, whereas fanning movements and water evaporation are utilized to decrease nest temperatures during summer months (Wilson, 1971).

Temperature regulation is also present in the nests of social wasps and ants. Colonies of Polistes, Vespa and Vespula have all been shown to utilize both fanning and water transport into the nest to decrease nest temperatures (Gaul, 1952) and
metabolic heat to raise them (Cloudsley-Thompson, 1964). The interior nest temperature of *Vespula vulgaris*, for example, can be maintained at 26°C when outside temperatures are as low as 9°C (Himmer, 1932; Wilson, 1966). Ants and termites are also able to regulate the environment of their nests (Andrews, 1927; Cloudsley-Thompson, 1970; Lindauer, 1954b; Luscher, 1961; Scherba, 1962; Steiner, 1929; Wilson, 1971). In addition, since most of these species nest beneath the surface of the soil, fluctuations in humidity and temperatures are minimized throughout the year.

It is important to note that although the previous discussion has been mainly concerned with high temperatures, many invertebrates are affected by and actively respond to falling temperatures (Clarke, 1967; Cloudsley-Thompson, 1962a; Kirchner, 1973; McWhinnie, 1967; Salt, 1958, 1969). This has been verified experimentally for millipedes (Cloudsley-Thompson, 1970), Woodlice (Edney, 1957, 1967), arachnids (Cloudsley-Thompson, 1971; Kirchner, 1973) and insects (Clarke, 1967; Cloudsley-Thompson, 1964; Kerkut and Taylor, 1958; Mellanby, 1939; Uvarov, 1931, Wellington, 1949; Wigglesworth, 1965). Several species of molluscs are extremely sensitive to falling temperatures and are able to detect changes as slight as 0.1°C/hr (Cloudsley-Thompson, 1970). Although extremes of cold are actively avoided, gradual falling temperatures generally produce only minor reactions among invertebrates (Fraenkel and
Gunn, 1961). Frequently, gradual cooling will decrease locomotor activity, which results in ectotherms becoming trapped in the cooler areas of a temperature gradient chamber (Cloudsley-Thompson, 1970; Deal, 1941; Grahm et al., 1965; Mellanby, 1939). Similar behavioral and physiological mechanisms to those used for adjusting to high temperatures are also utilized by arthropods in adjusting to low temperatures (Dainton, 1954; Heinrich, 1973; Salt, 1969; Scholander et al., 1953). The active selection of a favorable microhabitat is essential in escaping the lethal effects of low temperature extremes. Many insect species, for example, will burrow into the tissues of plants (Cloudsley-Thompson, 1970; Wellington, 1949) or beneath the surface of the soil (Allee et al., 1949; Odum, 1971; Uvarov, 1931) in order to escape extremes of cold. Arctic and subarctic arthropods have developed extensive sun-basking habits which effectively raise body temperatures 3° - 8°C above ambient temperatures (Bursell, 1964a; Cloudsley-Thompson, 1970; Prosser, 1955). Basking activities are also found in temperate and desert species during early morning hours when ambient temperatures are cooler (Clarke, 1960; Cloudsley-Thompson, 1962b; Fraenkel and Gunn, 1961).
Temperature Preferendum Studies

Initial experiments concerning temperature relationships of arthropods are mainly concerned with establishing the temperature preferences of these animals (Adams, 1937; Almquist, 1970; Deal, 1941; Gunn, 1942). Within the viable temperature range of a given species there is an ability to choose a preferred temperature. The temperature preferendum is defined as the temperature range to which an organism will move when exposed to a temperature gradient (Deal, 1941; Howe, 1951). Previous experiments on temperature preferences of terrestrial arthropods have been confounded by the fact that a gradient in temperature is also accompanied by a gradient in the relative humidity of the surrounding air. A great majority of the earlier investigations were performed without adequate relative humidity controls so that many of the effects attributed to temperature may in fact be due to differences in relative humidity (Buxton, 1931; Cloudsley-Thompson, 1970. Stringent humidity controls became available only in more recent years with the use of saturated salt solutions (O'Brien, 1948; Richardson and Malthus, 1955; Winston and Bates, 1960) and sophisticated environmental chambers (Grahm, 1965; Grahm et al., 1965; Henson, 1958). It is therefore important to emphasize that any investigation concerning the temperature relationships of an animal must take into consideration the concomitant moisture relationships as well.
Another point worth emphasizing is that the preferred temperature of an organism is often affected by interacting factors such as internal and external conditions and the previous thermal history of the organism in question (Madge, 1961). Thus the preferred temperature of an animal is rarely an absolute value but rather a range of temperatures (Cloudsley-Thompson, 1970; Deal, 1941; Uvarov, 1931). Most of the early investigations concerning the responses of invertebrates to temperature involved apparatuses which were provided with gradients in floor temperature and the resulting air gradients were crudely extrapolated from these floor gradients (Blake, 1970; Buxton, 1932; Campbell, 1937; Deal, 1941; Fulton, 1928; Gunn and Kennedy, 1936). Shelford and Deere (1913) constructed a rectangular chamber through which currents of air at different temperatures and humidities were passed. The moisture content of the air was designated as either moist (passed over water) or dry (passed over calcium carbonate). Stringent humidity controls were lacking. Other investigators used a metal bar which was cooled and heated at opposite ends respectively thus resulting in a gradient of temperatures along the bar (Adams, 1937; Gunn and Causeway, 1938; Gunn and Pielou, 1940; Herter, 1923, 1924, 1926; Martini, 1918; Thomsen and Thomsen, 1937; Thomson, 1938). Herter (1924) introduced a glass housing around this metal bar apparatus thereby achieving a more uniform experimental environment. The general objective
of these temperature gradient chambers is to ascertain the
temperature at which an organism will aggregate when provided
with a range of temperatures (Deal, 1941; Fraenkel and Gunn,
1961; Madge, 1961; Smart, 1935). Larvae of **Musca domestica**
have been shown to select a temperature range of 30° - 37°C
(Thomson, 1938) during their active feeding stages of develop-
ment. Shortly before pupation, however, they select substrate
temperatures closer to 15°C. Larvae of several species of
dung flies exhibit a temperature preferendum of 24° - 29°C
(Thomsen and Thomsen, 1937). Nicholson (1934) found that the
thermal preference of the blowfly, **Calliphora stygia**, a cool-
weather species, was 20° - 25°C, while that of **Lucilia cuprina**,
which inhabits warmer regions, was 30° - 32°C. An exhaustive
study on the temperature preferendum of 23 species of insects
was carried out by Deal (1941). In general, it was found that
insect species demonstrate temperature preferences for fairly
wide ranges of temperature rather than for specific absolute
temperatures. More recent investigations have supported this
conclusion (Allee et al., 1949; Burnett, 1956; Bursell, 1964a).
The work of Deal (1941) and earlier investigators has been
criticized by several authors because of the lack of stringent
humidity controls (Clarke, 1967; Cloudsley-Thompson, 1970;
Waterhouse, 1951; Wellington, 1949). It has been shown that
several insect species do not react appreciably to changes in
ambient temperature but rather to the evaporative power of the
air (Buxton, 1932; Bursell, 1964b; Chapman, 1971; Mellanby, 1932; Uvarov, 1931; Wellington, 1949; Wigglesworth, 1965). Therefore, in the absence of stringent humidity controls, effects attributed to changes in temperature may in fact be due to differences in the relative humidity of the air.

The thermal preference of insects and other arthropods can also be a function of the developmental stage of the animal (Agrell, 1964; Alexander and Ewer, 1958; Bodenheimer, 1929; Bongers and Weismann, 1971; Bowler, 1967; Bursell, 1964a; Seymour and Vinegar, 1973). For example, the young nymphs of Schistocerca gregaria exhibit a temperature preferendum of 27° - 29°C which increases to 36° for older nymphs (Bodenheimer, 1929, 1934; Cloudsley-Thompson, 1970).

Optimum and Lethal Temperatures

Temperature preferendum investigations on arthropods were followed by determinations of the optimum temperature and lower and upper lethal temperatures (thermal death points). The optimum temperature of an insect is defined as the mean temperature at which the greatest number of insects are reproduced in a given time period (Deal, 1941; Ludwig, 1956; Uvarov, 1931). The lower and upper lethal temperatures are the low and high temperatures, respectively, at which a specified percentage of animals die within a given period of exposure (Baldwin, 1954; Baldwin and House, 1954; Beattie, 1928; Cowles, 1962; Fraenkel
and Herford, 1940; Heber et al., 1973). For example, the LT$_{50}$ is the temperature at which 50% of the animals die within a given time period. Lethal temperatures vary considerably among terrestrial invertebrates. For an exposure period of 24 hours, the upper lethal temperature for *Lumbricus terrestris* (Annelida) is 29°C (Cloudsley-Thompson, 1970; Heimburger, 1924). It is 43°C for the mollusc, *Helix pomatia* (Edney, 1957), 37.5°C for the isopod, *Armadillidium vulgare* (Cloudsley-Thompson, 1971; Madge, 1961) and 38°C for the diplopod, *Glomeris marginata* (Crozier, 1924; Edney, 1954b). Upper lethal temperatures for a 24 hour exposure period have been shown to range from 39° - 50°C for arachnids (Alexander and Ewer, 1958; Cloudsley-Thompson, 1959, 1962b, 1964, 1970, 1971; Kirchner, 1973; Madge, 1961) and from 31° - 60°C for insects (Altman and Dittmer, 1973; Bowler, 1967; Cloudsley-Thompson, 1970, 1971; Currie, 1967; Edwards, 1958; Free and Spencer-Booth, 1962; Fulton, 1928; Howe, 1956; Mellanby, 1954a; Uvarov, 1931; Wellington, 1949). The lower lethal temperatures of arthropods are also quite variable and fall within a range of -30° - 0°C (Altman and Dittmer, 1973; Kirchner, 1973).

Lethal temperature determinations were then followed by investigations on the relative ability of arthropods to maintain activity at high and low temperatures. Heat-rigor and chill-coma determinations were used as criteria for assessing levels of activity. The heat-rigor temperature is defined as
the high temperature above which responses to stimuli fail to occur (Cloudsley-Thompson, 1962b; Cowles, 1962). The chill-coma value refers to the low temperature below which responses to stimuli fail to occur (Mutchmor and Richards, 1961; Staszak and Mutchmor, 1973a, 1973b). These inactivations are reversible when favorable temperatures are resumed (Mutchmor, 1967). In fact, chill-coma was originally described as the reversible rigor caused by low temperatures (Semper, 1881).

It is important to emphasize that chill-coma and heat-rigor temperatures, like preferred temperatures, are a function of the previous thermal history of the organism (Colhoun, 1960; Mellanby, 1939, 1954a; Staszak and Mutchmor, 1973a). In the assassin bug, *Rhodnius prolixus*, for example, when the rearing temperatures are 36°C, 30°C, or 17°C, the concomitant chill-coma temperatures are 12°C, 10.5°C and 8.6°C (Mellanby, 1939). The relationship between temperature and the duration of exposure required to produce chill-coma and heat-rigor in various arthropods has been investigated and discussed by Colhoun (1954, 1956, 1960), Rao and Bullock (1954), Dehnal and Segal (1956), Cloudsley-Thompson (1961, 1964), Free and Spencer-Booth (1961), Mutchmor and Richards (1961), Prosser (1961), and Kirchner (1973).
Reproductive Physiology

Lethal temperature determinations were followed by extensive studies of the effects of temperature on biological parameters such as reproduction, development, longevity, survival capacity and metabolic activities. Extremes of temperature can profoundly affect the reproductive physiology of arthropods (Chapman, 1971; Wigglesworth, 1965). In addition, the range of temperatures over which reproduction can effectively take place is a rather limited one (Andrewartha and Birch, 1954; Bursell, 1964a) and varies from species to species (Burnett, 1956). *Anopheles quadrimaculatus* females fail to oviposit at temperatures below 12°C, with the optimum temperature being approximately 23°C (Agrell, 1964). Males of *Aphytis linguanensis* (Hymenoptera) become sterile after brief exposure to temperatures below 1°C (Bursell, 1964a; Salt, 1936, 1969). Sterility takes place after short exposures to 32°C in several species of *Drosophila* (Cloudsley-Thompson, 1962a). Some species of insects are able to lay eggs over a fairly wide range of temperatures. For example, the aphid, *Toxoptera graminum*, will lay eggs at temperatures ranging from 5° - 30°C (Bursell, 1964a); the ptinid beetle, *Ptinus tectus* will oviposit over a range of 5° - 30°C (Bursell, 1964a; Coombs and Woodrffe, 1962). Within the range of temperatures at which reproduction is possible, rates of oviposition can also be temperature-dependent (Burnett, 1956; Chapman, 1971).
Generally, it has been found that the maximum rate of oviposition occurs at temperatures approaching the upper limit for reproduction and decreases sharply at higher temperatures and more slowly at lower temperatures (Bursell, 1964a; Cloudsley-Thompson, 1964; Goonewardene and Townshend, 1974; Mueller and Stern, 1973).

**Developmental Processes**

Temperature also affects developmental processes which, as a rule, can occur only within a relatively narrow temperature range. Previous investigations on developmental threshold temperatures have been extensive (Agrell, 1964; Burges, 1962; Bursell, 1964a; Currie, 1967; Hafeez and Chapman, 1966; Hagstrum and Leach, 1973; Hamilton, 1936; Mueller and Stern, 1973; Richards, 1964; Stoner and Weeks, 1974; Toba et al., 1973; Wilson and Miner, 1969). Not only may the rate of development be affected but also the longevity of the adults and the duration of various pre-adult developmental stages (Agrell, 1964; Amos and Morley, 1971; Chapman, 1971; Nayar, 1972; Smith, 1958; Wigglesworth, 1965). Another interesting developmental parameter with respect to temperature, that has been elucidated in recent years, is the fact that changes occur in temperature tolerance during the developmental stages of insects (Bowler, 1967; Davison, 1969, 1971; Hollingsworth and Bowler, 1966) and other arthropods (Clarke, 1967; Cloudsley-Thompson, 1964, 1970; Edney, 1967; Kirchner, 1973).
Survival Capacity

The effects of temperature on mortality and survival capacity have also been extensively investigated (Allee et al., 1949; Bacot and Martin, 1924; Blumberg, 1971; Bodenheimer, 1929, 1934; Clark et al., 1967; Davidson and Andrewartha, 1948; Decker and Andre, 1936; Edwards, 1964; Haverty and Nutting, 1974; Kehat, 1967; Klomp and Gruys, 1965; Morris, 1965; Mueller and Stern, 1973; Nicholson, 1933; Roth and Willis, 1951a, 1951b; Schwalbe et al., 1973; Sweetman, 1933; Uvarov, 1931). Generally, these investigations have shown that survival capacity and mortality are markedly affected by interactions of temperature and relative humidity as well as by the moisture content of the medium in which the animal may be found. The survival capacity or resistance to combinations of temperature and relative humidity extremes has been systematically studied in detail in only a relatively few insect and other arthropod species (Bailey, 1969; Belehradek, 1935; Blumberg, 1971; Cloudsley-Thompson, 1962b; Free and Spencer-Booth, 1962; Grahm, 1958; Howe, 1956, 1960, 1968; Kehat, 1967; Kirchner, 1973; Lefkovitch and Currie, 1967; Mellenby, 1932, 1954a; Nuttall, 1970; Roth and Willis, 1950; Salt, 1969; Smith, 1957; Watters, 1966; Whitney, 1939). Kehat (1967), for example, found that at high temperatures (43° - 45°C), in contrast to preferred temperature conditions (32°C), relative humidity exerted a marked effect on the survivorship of adults of the
coccinellid, *Pharoscyphum numidicus*. At long periods of exposure, mortality was higher at lower humidity levels (32%) for this species. However, within the normal temperature range of the animal (24° - 34°C), humidity extremes as low as 10% had no deleterious effects on the survival capacity of *P. numidicus*. Studies on the survival capacity of the cybocephalid beetles, *Cybocephalus nigriceps* and *C. micans* (Blumberg, 1971) show that the effects of humidity on survival are more pronounced at extremes of temperature and conversely, temperature effects are more pronounced at extremes of humidity. This is an excellent example of factor interaction (Odum, 1971). Similar findings have been reported for several insect species which include the curculionids, *Hypera postica* (Sweetman, 1933) and *Sitophilus granarius* (Bailey, 1969; Howe and Hole, 1968; Robinson, 1926; Smerka and Hodson, 1959; Surtees, 1964b), the silvanid beetle, *Oryzaephilus surinamensis* (Amos, 1968), the ptinid beetle, *Ptinus tectus* (Bentley, 1944), the flour beetles, *Tribolium castaneum* (Howe, 1956) and *T. confusum* (Howe, 1960; Watters, 1966), the anobiid, *Lasioderm serricorne* (Lefkovitch and Currie, 1967), the dermestid beetle, *Trogoderma granarium* (Schwalbe et al., 1973; Yinon and Shulov, 1969), the noctuid pine looper, *Bupalus piniarius* (Klomp and Gruys, 1965) and the tortricid, *Choristoneura fumiferana* (Morris, 1965; Wellington, 1949), as well as the arachnids, *Clubiona similis*, *Euphryus frontalis*, *Phrurolithus festivus* and *Stemonyphantes lineatus* (Almquist, 1970), *Theridion saxatile* (Norgaard, 1956), the lycosid spiders,
Pirata piraticus and Lycosa pullata (Norgaard, 1951), several species of linyphids, philodromids, tetragrathids and theridiids (Kirchner, 1973), the earth mite, Halotydeus destructor (Cloudsley-Thompson, 1964), and the centipedes, Scolopendra polymorpha and Lithobius sp. (Cloudsley-Thompson and Crawford, 1970).

In recent years there has been a shift in interest to the molecular mechanisms involved in the temperature adaptations of ectotherms. As was previously mentioned, the temperature preferendum as well as the upper and lower lethal temperature of an ectotherm is directly dependent on the previous thermal history of the organism in question. Acclimation of ectotherms at low and high temperatures is known to change the thermal tolerance of these animals (Baldwin and Riordan, 1956; Bateman, 1967; Bullock, 1955; Fry, 1958, 1967; Hochachka, 1966; Mutchmor, 1967; Newell, 1973) as well as the chill-coma (Baust and Miller, 1972; Hochachka, 1971; Mellanby, 1939; Mutchmor and Richards, 1961; Staszak and Mutchmor, 1973a) and heat-rigor (Baldwin and House, 1954; Cloudsley-Thompson, 1970; Cowles, 1962; Holzman and MacManus, 1973; Scholander et al., 1953) temperatures. Moreover, acclimation not only affects the upper and lower lethal temperatures, but also the period of exposure to a lethal temperature that an organism can tolerate (Andrewartha

The ability of ectotherms to adjust to temperature changes in the environment is predicated upon the ability to maintain metabolic processes, despite extremes of temperature. This ability has enabled such organisms to survive in both xeric and arctic environments (Bateman, 1967; Bullock, 1955; Dehnal and Segal, 1956; Fry, 1967; Hochachka and Somero, 1968; Hochachka and Lewis, 1970; Kimura, 1968; Meats, 1973; Mutchmor and Richards, 1961; Newell, 1966; Precht, 1958; Prosser, 1961; Rao, 1967; Somero and Hochachka, 1971; Ushakov, 1964). The metabolic processes of ectotherms, due to their relative inefficiency to adjust their body temperature as compared to homeotherms, are more directly dependent upon the environmental temperature (Mutchmor, 1967; Prosser, 1961; Wieser, 1973). In this case, environmental temperatures directly influence enzyme reaction rates and the adaptive mechanisms of the organism must be initiated at this level. As Hochachka (1971) points out, the metabolic activities of many ectotherms are intimately adjusted to the thermal regime of their normal habitat. For example, the rate of metabolism of cold-weather ectotherms at approximately 0°C is as high as that of temperate ectotherms at 15° - 25°C (Cloudsley-Thompson, 1970; Fry, 1967; Hochachka, 1966, 1971; Marzusch, 1952; McFarlane and McLusky, 1972; Mutchmor,
1967; Mutchmor and Richards, 1961; Rao and Bullock, 1954; Somero et al., 1968). This type of adaptive metabolic functioning occurs over evolutionary time and is therefore distinct from the shorter seasonal acclimatizations found in many ectotherms (Hochachka, 1966).

Metabolic adaptations of ectotherms to changes in temperature are expressed by shifts in metabolic rate-temperature curves either to the right for high temperatures or to the left for low temperatures (Belenhraděk, 1957; Bullock, 1955; Cloudsley-Thompson, 1970; Hochachka, 1971; Mutchmor and Richards, 1961; Precht, 1958; Prosser, 1961; Richards, 1963; Silverthorn, 1973; Vernberg and Moreira, 1974) or by changes in the slope ($Q_{10}$) of this curve (Anderson and Mutchmor, 1971; Clarke, 1967; Mutchmor, 1967; Newell, 1966; Rao and Bullock, 1954; Richards, 1956; Valen, 1958). In addition, acclimation responses from cold-adapted organisms are of greater magnitude than those of warm-adapted animals (Fry and Hochachka, 1970; Holzman and MacManus, 1973; Mutchmor, 1967; Somero et al., 1968). It is important to point out that three basic types of temperature adaptations can be distinguished on the basis of time: immediate thermal compensation, thermal acclimation and evolutionary adaptation (Hochachka, 1971).

The general effect of a reduction in temperature is to decrease the rate of metabolic activities (Clarke, 1967; Prosser, 1961). A notable exception is the brief stimulation
of the central nervous system by reduced temperatures which results in locomotor activity in slugs, millipedes and other arthropods (Cloudsley-Thompson, 1962a, 1970; Kerkut and Taylor, 1958). Presumably this effect occurs over a limited temperature range only, and is associated with nocturnal activity (Cloudsley-Thompson, 1959; Marler and Hamilton, 1966). Conversely, a rise in temperature increases the metabolic rate of the organism (Chapman, 1971; Prosser, 1961).

Examples of thermal acclimation among terrestrial invertebrates are not nearly as extensive as those for aquatic ectotherms. The upper lethal temperature of the annelid, Pheretima sp., was shown to increase 0.3° per 1°C rise in acclimation temperature (Cloudsley-Thompson, 1970). Gelineo and Kolendig (1953) maintained one group of snails (Helix pomantia) at 6°C and another group at 20°C for a period of two months. Following this, the oxygen consumption of both groups was measured at 6° and 30°C. The oxygen consumption of the cold-acclimated group was 40% higher at 30°C and 19% higher at 6°C than that of the warm-acclimated group. In the slug, Arion circumscriptus, acclimated to temperatures of 5° - 25°C, it was found that the average metabolic rate as indicated in measurements of oxygen consumption or direct calorimetry was 1 - 1.5% lower per 1°C rise in acclimation temperature (Roy, 1963). Also, when the logarithm of oxygen consumption was plotted against the logarithm of body weight the resultant regression
plot for warm-acclimated slugs was below (to the right) that of the cold-acclimated group. In addition, the slope for the warm-acclimated group was less so that the distance between the two plots was greater on the right side of the graph where the larger specimens were represented thereby suggesting that an increase in acclimation temperature decreases the metabolic rate of a greater extent in larger specimens. Similar results have been more numerously reported for aquatic ectotherms (Barkes, 1959; Brett, 1956; Bullock, 1955; Cocking, 1959; Fry, 1958, 1967; Hochachka, 1971; Kanungo and Prosser, 1960; Krog, 1954; McLeese, 1956; Newell, 1973; Prosser, 1961; Roberts, 1957; Segal, 1956; Tashian and Ray, 1957; Vernberg and Moreira, 1974; Whitney, 1939).

Temperature acclimation is more systematically documented for the Arthropoda than for the annelids and molluscs (Flemister, 1964). The upper lethal temperature ($LT_{50}$) for the isopod, Armadillidium vulgare at a rearing temperature of 30°C is 41.6°C (Edney, 1954a). The $LT_{50}$ for the wood louse, Porcellio laevis, in saturated air at a rearing temperature of 30°C is also 41°C, while the $LT_{50}$ for preconditioned animals at 10°C falls to 38.3°C (Edney, 1957). The lower lethal temperatures as well as the metabolic rates of these species were also affected (Cloudsley-Thompson, 1970). It has been shown that the heat-coma and thermal death points of several adult insect species (Lucilia sericata, Pediculus humanus, Tenebrio molitor,
Xenopsylla cheopis) are significantly altered by the temperature at which these insects are kept prior to LT_{50} determinations (Mellanby, 1954b). Acclimation at temperatures approaching the upper lethal temperature for a period of 24 hours increased the period of exposure necessary for death by 4 hours in the scorpion, Leiurus quinquestriatus, and in the tenebrionid beetles, Ocnera hispida, and Pimelia grandis (Cloudsley-Thompson, 1962b). Acclimation of Pseudosarcophaga affinis (Diptera) larvae for only 2 hours at 39°C increases the period of exposure required to yield 50% mortality by nearly 6 hours as compared to larvae reared at 23°C (House et al., 1958).

Free and Spencer-Booth (1962) found that the acclimation of honeybees to 35°C enhanced their survival capacity at temperatures as high as 47°C as compared with bees acclimated to 20°C.

With respect to cold temperature compensation, investigations generally involve the effects of cold-acclimation on the chill-coma temperature of the organism in question. Mellanby (1939) reported that individuals of several insect species including Blatta orientalis, Cimex lectularius, and Rhodnius prolixus, that were acclimated to warm temperatures, became immobilized at higher chill-coma temperatures than individuals acclimated to colder conditions. Individuals of Blatella germanica maintained at 15°C were able to sustain locomotor activity at temperatures of 8° - 12°C; these temperatures produced chill-coma in animals maintained at 35°C (Colhoun, 1954,
It is important to emphasize at this point that acclimation to cold is not a universal process and frequently is only possible within a narrow range of temperature (Newell, 1966; Precht, 1968; Prosser, 1961). In several species of insects, for example, no metabolic adaptation to unfavorable temperatures has yet been demonstrated. Several species of insects seem unable to modify metabolic processes in relation to unfavorable temperatures; in this sense they are strictly poikilothermic (Edwards, 1958).

Short-term seasonal acclimitizations are also shown by terrestrial invertebrates (Baldwin, 1954; Barkes, 1959; Bateman, 1967; Colhoun, 1954, 1960; Dehnal and Segal, 1956; Gelineo and Kolendig, 1953; Krog, 1954; Marzusch, 1952b; Meats, 1973; Newell, 1973; Precht, 1968; Rao and Bullock, 1954). Snails of the genus Helix, for example, show seasonal shifts in heart rates when compared under standard laboratory conditions (Crozier and Stier, 1926). The temperature characteristic for heart rate in the gastropod, Limax maximus, is 11,500 calories during the winter months and 16,200 calories during the summer months (Crozier and Stier, 1926; Prosser, 1955). Edney (1957) reported differences of 3° and 2.4°C, respectively, between the summer and winter lethal temperatures of Porcellio laevis and Armadillidium vulgare. Similar seasonal shifts in lethal temperatures have been verified for several species of millipedes (Cloudsley-Thompson, 1959), centipedes (Cloudsley-

It is evident, therefore, that many invertebrates exhibit changes in preferred temperature, lethal temperature, chill-coma and heat-rigor temperatures and rates of metabolic processes when conditioned to different temperature regimes. In general, ectotherms respond to abrupt increases or decreases in temperature by raising or lowering metabolic rates. This initial rate response to changes in environmental temperature may proceed for seconds or several minutes (Bullock, 1955; Precht, 1968; Prosser, 1961). This initial response phase is usually followed by a period of stabilization of metabolic rates during which most $Q_{10}$ determinations are made (Clarke, 1967). Frequently, if the animal is returned to the original temperature during this stabilization period, the metabolic rate also returns to its initial level (Belehradek, 1957; Burkett, 1962; Fry, 1958, 1967; Herter, 1953; Hochachka, 1967; Meyer, 1974; Prosser, 1961). If the animal is maintained at the altered temperature for a period of hours or days, the metabolic rate functions begin to show certain levels of
compensation or, in other words, become acclimated (Anderson and Mutchmor, 1971; Bullock, 1955; Chapman, 1971; Fry, 1967; Fry and Hochachka, 1970; Holzman and MacManus, 1973; Mutchmor, 1967; Newell, 1966; Rao, 1967; Roy, 1963; Silverthorn, 1973; Vernberg and Moreira, 1974). Following this period of thermal conditioning, if the organism is once again exposed to the original temperature, the metabolic rate functions will generally persist for sometime at the lower or higher level according to the direction of acclimation (Hochachka, 1967, 1971; Precht et al., 1955).

These compensatory responses of various metabolic rate functions shown by ectotherms to changes in environmental temperatures are reflected at the molecular level by the acclimation of enzyme activity (Anderson and Mutchmor, 1971; Fry and Hochachka, 1970; Hochachka, 1971; Hochachka and Somero, 1968; Hochachka and Lewis, 1970; Moreland and Watts, 1967; Richards, 1963; Somero and Hochachka, 1971; Wieser, 1973). It is well known that species with wide geographical ranges generally develop regionally adapted populations called ecotypes which have adjusted their optimum limits of tolerance to local conditions. These local compensations are usually accomplished through physiological mechanisms which are reflected by shifts in enzyme-substrate relationships (Bullock, 1955; Precht, 1968; Somero and Hochachka, 1971). Somero (1969) has suggested that immediate temperature compensations are
accomplished by an inverse relationship between temperature and enzyme-substrate affinity, while longer-term evolutionary adaptations are more likely to involve changes in the enzyme-substrate affinity itself.

Traditionally, the effects of temperature on metabolic activities, as reflected by enzyme reaction rates, have been assessed according to Arrhenius plots and $Q_{10}$ determinations (Giese, 1968; Mutchmor and Richards, 1961; Rao and Bullock, 1954; Valen, 1958). More recently, the emphasis has been on kinetics of enzyme-catalyzed metabolic reactions.

It has been suggested by some investigators that the basic mechanism of thermal acclimation in ectotherms is predicated upon the ability of the organism to synthesize lower or higher quantities of enzymes in order to compensate for increases or decreases in temperature (Caldwell and Vernberg, 1970; Cloudsley-Thompson, 1970; Ekberg, 1958; Fry, 1967; Rao, 1962; Roots and Prosser, 1962). However, the emphasis in these studies has been on the measurement of maximum catalytic activities (Fry and Hochachaka, 1970) without any reference to the conformational properties of the enzymes responsible for metabolic compensations exhibited by ectotherms to changing temperatures (Behrisch, 1972; Haites et al., 1972; Hochachka and Lewis, 1970; Masters and Holmes, 1974; Roberts, 1966; Somero, 1969a; Wernik and Kunnemann, 1973; Wieser, 1973). The selective or functional advantage of producing increased or decreased amounts of enzymes to compensate for changes in
temperature has not been entirely apparent and the search for alternative mechanisms has intensified.


A number of different enzymes have been found to exist in multiple molecular forms within a given species. These multiple molecular forms can be separated by electrophoretic methods, which indicates that they differ in net electrical charge. Multiple molecular forms of electrophoretically distinct enzymes with identical function are called isozymes; each isozyme has its own characteristic $K_m$ value (Chiu et al., 1972; Decker and Rau, 1973; Fritz, 1967; Funakoshi and Deutsch, 1971; Grell et al., 1965; Harris, 1969; Kaplan et al., 1960; Lehninger, 1970; Markert, 1968; Pfleiderer and Zwilling, 1972; Shaw, 1969; Watts, 1968; Webb, 1964; Wilkinson, 1970).

The relevance of isozyme induction as a mechanism of thermal compensation was initially recognized in studies concerning the role of lactate dehydrogenase (LDH) isozymes in the thermal acclimation of goldfish (Hochachka, 1965) and
trout (Hochachka, 1967; Hochachka and Somero, 1968; Hochachka and Lewis, 1970). It was found that during cold acclimation, new isozymic forms of LDH can be found which have different kinetic properties from the LDH forms present at normal temperatures. The LDH isozymes of cold-acclimated animals are characterized by minimal $K_m$ values and higher affinities for substrate at lower temperatures. Similar findings have been reported for pyruvate kinase (PyK) from trout muscle (Somero, 1969a). PyK is responsible for the transfer of a phosphate group from phosphopyruvate to ADP in the glycolytic sequence, thereby forming pyruvate (White et al., 1968). Muscle PyK from warm-acclimated animals is characterized by minimal $K_m$ constants in the range of 15° - 20°C. Acclimation at cold temperatures induces a new PyK isozyme with minimal $K_m$ constants in the range of 5° - 7°C. Two isozymic forms of acetylcholinesterase have been identified from trout brain, one occurring only in warm-acclimated animals (22°C); the other in cold-acclimated fish (2°C). Both forms are present in the same animal at an intermediate temperature of 12°C (Baldwin and Hochachka, 1970). Choline acetyltransferase occurs in two forms in the goldfish (Fry and Hochachka, 1970). The warm-acclimated enzyme has a higher $K_m$ value at low temperatures. Additional LDH isozyme inductions in response to changes in temperature have been reported by several investigators (Agostini et al., 1966; Fritz and Jacobson, 1965;
Fry and Hochachka, 1970; Hochachka, 1965, 1966; Latner and Skillen, 1968; Vessel and Yielding, 1966; Wernick and Kunnemann, 1973; Zondag, 1963). Temperature-induced isozymic forms of esterases (Burns and Johnson, 1970; Townson, 1969) transaminases (Decker and Rau, 1963; Otsuka, 1965), carbonic anhydrases (Funakoshi and Deutsch, 1971), and hexokinases (Katzen et al., 1970) have also been verified. Other workers have reported similar findings for temperature-induced isozymes in reptiles (Aleksiuk, 1971; Roberts, 1966; Wieser, 1973), amphibians (Fitzpatrick et al., 1971; Hensen, 1972; Kasbohm, 1967), insects (Beckman and Johnson, 1964; Burns and Johnson, 1970; Fitzsimmons and Doherty, 1970; Grell et al., 1965; Haites et al., 1972; Johnson, 1971; Masters and Holmes, 1972; Tanabe et al., 1970; Townson, 1969; Wagner and Selander, 1974; Wieser, 1973), crustaceans (Behrisch, 1972; Chesseman et al., 1967; Haites et al., 1972; Newell and Northcroft, 1967), molluscs (Haites et al., 1972; Moreland and Watts, 1967; Roberts, 1966) and annelids (Campbell, 1965; Mangum, 1972; Newell and Northcroft, 1967). The careful analysis of these findings suggests that the basic process of temperature acclimation is not the modulation in quantity of enzymes present but rather the synthesis of new enzyme variants (isozymes) which are functionally better adapted and hence facilitate metabolic activities so that the organism may remain active at the new temperature regime (Aleksiuk, 1971;
Baldwin and Hochachka, 1970; Fry and Hochachka, 1970;
Hochachka and Somero, 1968; Newell, 1973; Pandey, 1972; Somero,
1969b; Somero and Hochachka, 1971; Tribe and Bowler, 1968;
METHODS AND MATERIALS

General

Experiments were conducted to determine the effects of temperature, moisture (relative humidity) and thermal acclimation on the survival capacity, changes in larval temperature tolerance, longevity of adults, oviposition rate, locomotor activity and dispersion behavior of the darkling beetle, *Tenebrio molitor*. The insects were taken from a laboratory stock culture maintained at 25°C and 75% relative humidity, and kept under conditions of constant darkness. The beetles were reared in a cereal medium consisting of 70% wheat bran and 30% corn meal.

Survival Capacity

Experiments were carried out to investigate the survival capacity of *T. molitor* as a function of temperature, relative humidity and length of exposure. These studies were conducted in a Freas Model 805 Incubator at temperatures of 10°, 25° and 35°C, and at relative humidities of 12, 52, 75 and 98%. All tests were conducted under conditions of constant darkness. The various humidity values were obtained through the use of saturated salt solutions as reported by Winston and Bates (1960): $K_2SO_4$ yielding 98%, NaCl yielding 75%, sucrose plus urea yielding 52%, and LiCl $\cdot$ H2O yielding 12% relative humidity (r.h.). These solutions were placed in the bottom of
one-quart air-tight plastic humidity chambers, to a depth of 4 cm. These humidity chambers were provided with a screen grid platform (Kehat, 1967) positioned 2 cm above the salt solution. With the lid on the container, the desired humidity value was reached within 30 min. Five 5-ml plastic beakers, provided with cereal identical to that used in the rearing colonies, were placed on the grid platform and provided holding containers for the experimental animals during test procedures. Investigations were conducted at all developmental stages.

The survival capacity of the egg stage was determined as a function of the amount of water uptake that occurred throughout embryological development. The eggs used in this study were obtained by removing adult females from the rearing colonies and isolating them in 5-ml plastic beakers provided with cereal. These containers were checked daily and the eggs were collected as soon as possible after oviposition. Twenty eggs were also placed in 5-ml plastic beakers provided with blotting paper. These beakers were then placed into the test containers and allowed to develop at temperatures of 10°, 25° and 35°C; at each test temperature water uptake was recorded under relatively dry (12% r.h.) and moist (75% r.h.) conditions. Due to the early mortality (1-3 days) of approximately 50% of the sample, apparently not attributable to the treatment conditions, data from 10 developing eggs was recorded and utilized in the analysis. Individual eggs were removed from
the test containers and weighed daily on a Christian Becker Model 109 microbalance, and then returned to the test container. This procedure was repeated throughout embryonic development until eclosion or mortality from desiccation took place.

The survival capacity of the larval stage was analyzed by investigating both younger and older individuals at various combinations of temperature, humidity and length of exposure. The larvae were divided into two developmental stages differentiated on the basis of larval length (Ernst and Mutchmor, 1969) and weight (Mellanby, 1932): young larvae (< 30 mg; 6-12 mm) and older larvae (> 100 mg; 25-30 mm). One hundred larvae were studied at each larval phase of development. Identical one-quart test containers were used for humidity chambers. Three Freas constant-temperature incubators were set at 10°, 25° or 35°C. For each temperature, 12 one-quart humidity chambers were used (3 at each humidity value of 12, 52, 75 and 98%). For each combination of temperature and relative humidity, exposure periods of 6, 12, 24 and 48 hours were investigated. Each humidity chamber housed 5 larvae, individually placed in 5-ml plastic beakers containing the appropriate cereal medium. Thus, at each replication, 20 larvae were tested at each temperature, humidity and length of exposure. Five replications yielded data for 100 young and older larvae. Following the prescribed period of exposure,
each larva was isolated in an individual holding container under conditions identical to those of the rearing colonies (25°C/75% r.h.). Survival capacity was determined by recording the percentage of insects surviving 48 hours after removal from test conditions (Blumberg, 1971; Kehat, 1967).

Identical procedures were employed for the pupal and adult stages of T. molitor. The survival capacity of the adults was again determined by the number surviving (out of 100) 48 hours after removal from test conditions, while that of the pupae was determined by recording the percentage of individuals successfully completing development and emerging as adults after removal from test conditions.

Changes in Larval Temperature Tolerance

Adult females were taken from the rearing colonies, isolated in 10-ml plastic containers provided with cereal and allowed to oviposit at 25°C/75% r.h. Eggs were then collected and allowed to develop. First instar larvae were isolated in 5-ml plastic containers for further development. Larval instars were identified by counting the number of exuvia. Ten larvae at each of the 27 larval instars were removed from rearing conditions and subjected to an upper stressful temperature of 42°C at relative humidities of 12, 75 and 98%. Changes in temperature tolerance were determined by recording the amount of time required for mortality to occur at each larval
instar. The humidity chambers used in this experiment were identical to those utilized in the survival capacity experiments.

**Longevity of Adults**

Individual pupae were obtained from the rearing colonies and sex determinations were performed as described by Halstead (1963). The sex of the pupae was determined by noting the morphology of the developing genital structures which are situated immediately posterior to the 7th visible abdominal sternite. In the male, there is a small but conspicuous swelling which protrudes from beneath the 7th Sternite and bears a pari of short, blunt papillae which are closely positioned on the mesal line. In the female, the homologous swelling is distinctly larger and bears a pair of widely diverging papillae. Pupae were placed in separate containers and allowed to develop at 25°C/75% r.h. Upon eclosion, the adult males and females were reared at all combinations of temperature (10°, 25° and 35°C) and relative humidity (12, 75 and 98%) and the effects of these physical parameters on their longevity expressed in days after pupal emergence was analyzed.

**Oviposition Rate**

Adult females were obtained from the rearing colonies and placed individually in humidity chambers provided with cereal.
Ten females were tested at identical combinations of temperature and relative humidity as those utilized in the longevity experiments. The mean number of eggs laid per female over a 48-hour period was used as the index of oviposition rate and the effects of temperature and relative humidity on this parameter were assessed.

**Locomotor Activity**

The effects of temperature and moisture on the locomotor activity of *T. molitor* larvae were assessed by recording the amount of time necessary for larvae to burrow completely into the cereal medium in order to avoid a light stimulus. The strong negative phototactic response of this species has been previously verified by several investigators (Cotton, 1950; Howard, 1955; Yinon, 1970). Larvae were placed in the humidity chambers and temperature cabinets at combinations of temperature (10°, 25° and 35°C), relative humidity (12 and 75%) and length of exposure (1, 6, 12 and 24 hours) and subsequently individual larvae were removed from the humidity chambers and placed on the surface of a cereal medium in a 50-ml glass container at 25°C. This container was positioned directly beneath a 22-watt cool fluorescent lamp which served as the light stimulus. The amount of time required for the larvae to burrow into the grain was recorded with a Lab-chron Model 1400 electric timer.
The effects of temperature and moisture on the locomotor activity of *T. molitor* adults were assessed by recording the ambulatory speed of these insects in a runway apparatus. The apparatus consisted of a straight runway constructed of opaque plexiglass. The length of the runway was 15 cm; the width was 1.5 cm and the height of the walls was 3 cm. One end of the runway was provided with a completely enclosed darkened chamber (Figure 1). Adults were subjected to identical combinations of temperature, moisture and length of exposure as were the larvae. Following preconditioning procedures, individual adults were removed from the humidity chambers and placed in the runway at point A (Figure 1-A) at 25°C. The time required for the insect to reach and enter the enclosed chamber (Figure 1-B) was recorded. A 22-watt fluorescent lamp was used as the aversive light stimulus and was placed directly over the runway apparatus.

**Dispersion Behavior**

Experiments were conducted to study the effects of temperature and thermal acclimation on the three-dimensional dispersion behavior of larval and adult *T. molitor*. The dispersion apparatus consisted of a wooden box (34-cm cube) which held 25 kg of a standard commercial grain mixture consisting of cracked corn, wheat and milo maize (Wertz Feed Products). The cube consisted of four individual vertical layers and was
Figure 1. Diagrammatic representation of the runway apparatus used to analyze the running speed of Tenebrio molitor adults. Adult insects were placed at point A and the amount of time required to traverse the runway and enter the darkened chamber (B) was recorded.
similar in design to the apparatus employed by Surtees (1964b). Each vertical layer was divided into 16 cubic compartments (Figure 2). When assembled, the vertical layers were confluent to one another. Horizontal grooves were present between each vertical layer and a thin formica sheet was inserted in these grooves in order to isolate one vertical layer from another. The cube was filled with grain and allowed to acclimate to test conditions for one week prior to investigation.

*T. molitor* larvae were obtained from the rearing colony (25°C) and tested for patterns of dispersion. The larvae were divided into the following temperature groups: (1) Group 1: larvae taken directly from the rearing colony (acclimated at 25°C) and tested for dispersion in the cube at 10°C (25°a/10°t); (2) Group 2: larvae taken from the rearing colony and tested at 25°C (25°a/25°t); (3) Group 3: larvae taken directly from the rearing colony and tested at 35°C (25°a/35°t); (4) Group 4: larvae were acclimated at 10°C for 24 hours and then tested at 10°C (10°a/10°t); (5) Group 5: larvae acclimated at 35°C for 24 hours and then tested at 35°C (35°a/35°t). Fifty larvae were tested at each temperature condition. The larvae were introduced into the bottom of the middle-top vertical layer of the cube. All vertical layers were provided with grain. The four vertical layers were then assembled and the formica sheets separating these layers removed. The cube
Figure 2. Diagrammatic representation of the three-dimensional dispersion apparatus which consisted of a wooden 34-cm cube. The cube was divisible into 4 vertical layers: top, middle-top, middle-bottom and bottom. These four vertical layers could be separated from each other by the insertion of a thin formica sheet between them. Each vertical layer was further subdivided into 16 cubical compartments by the insertion of a formica partition.
was then placed into a constant temperature cabinet at the prescribed test temperature. The dispersion pattern of the larvae was analyzed at time intervals of .5, 1, 2, 3 and 4 hours. After the prescribed time period, the cube was removed from the temperature cabinet and the vertical layers were isolated from each other by the insertion of the formica sheets thereby effectively stopping further vertical dispersion. The cube was then disassembled into the four component layers. A formica partition dividing each layer into 16 cubical compartments (Figure 2 and Figure 11) was immediately inserted into each vertical layer to stop any further horizontal dispersion. The insects and grain from all compartments in each vertical layer were collected with an Ace-Sycamore Model Al624 Vacuum Pump and isolated in individual containers for counting. Counts were recorded from all compartments in each vertical layer for all test conditions.

The procedures used to analyze the dispersion behavior of T. molitor adults were identical to those described for the larvae.

In order to obtain a reasonable estimate of the moisture conditions present in the cube, the moisture content of the grain was determined using an Ohaus Model 6000 moisture determination balance. Samples of grain were allowed to condition for one week at the test temperatures of 10°, 25° and 35°C, and a relative humidity of 75% which was obtained by placing trays of water at the bottom of the constant temperature cabinets.
RESULTS

Survival Capacity

The effects of temperature and moisture on the water uptake and ultimate survival capacity of *T. molitor* eggs are shown in Figure 3. Under relatively moist and optimal humidity conditions (75%), the degree of water uptake was significantly higher at 25°C (*F* = 187.03, *df* = 1/18, *p* < .001) and 35°C (*F* = 192.46, *df* = 1/18, *p* < .0001) than it was at 10°C. The rate of water uptake was more rapid during the first four days after oviposition at test temperatures of 25°C and 35°C. Embryological development was more rapid at 35°C than it was at 25°C (*F* = 226.73, *df* = 1/18, *p* < .0001). At 10°C, however, water uptake was minimal and embryological development was never completed.

Under relatively dry conditions (12%), as shown in Figure 3, the eggs of this species transpired water and decreased in weight at all test temperatures, although this effect was most severe at higher temperatures (35°C and 25°C). In all cases, the eggs progressively dehydrated and mortality due to desiccation resulted after 11-14 days. When examined under a dissecting scope, these dehydrated eggs appeared severely wrinkled, darkly colored and hardened. Under normal developing conditions the eggs remain translucent, ovid and soft. In order to ensure that mortality had occurred and that the desiccation was irreversible, several of the dehydrated eggs from
Figure 3. The mean water uptake in mg of the eggs of Tenebrio molitor as a function of temperature and relative humidity. Each data point represents the mean value of 10 eggs. Data was recorded throughout embryological development until eclosion (E) or desiccation (D) took place.
temperature were placed in containers under optimal conditions (25°C/75% r.h.). In all cases no further embryological development ensued.

Analysis of variance showed highly significant overall temperature and moisture effects on the daily water uptake of the eggs (F = 893.66, df = 5/18, p < .0001). The overall analysis of variance is shown in Table 1.

Table 1. Analysis of variance for the effects of temperature and moisture on daily water uptake of T. molitor eggs

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>ss</th>
<th>ms</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups (T°/r.h. %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numerator</td>
<td>5</td>
<td>130.57</td>
<td>43.525</td>
<td>9999.99*</td>
</tr>
<tr>
<td>Denominator</td>
<td>36</td>
<td>0.15</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numerator</td>
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<td>11.55</td>
<td>0.6421</td>
<td>893.66*</td>
</tr>
<tr>
<td>Residual</td>
<td>648</td>
<td>0.46</td>
<td>0.0007</td>
<td></td>
</tr>
<tr>
<td>Groups x Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numerator</td>
<td>90</td>
<td>21.20</td>
<td>0.3926</td>
<td>546.45*</td>
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<tr>
<td>Residual</td>
<td>648</td>
<td>0.46</td>
<td>0.0007</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at p < 0.0001.

The effects of temperature and moisture on the survival capacity of the larval (younger and older), pupal and adult stages of T. molitor are shown in Table 2 through Table 4 and in Figure 4. Analysis of variance showed highly significant overall effects of these test temperatures (F = 87.21, df = 2/36, p < .0001), relative humidities (F = 197.40, df = 3/36,
Table 2. Survival capacity of *Tenebrio molitor* life stages at 10°C and various combinations of relative humidity (12, 52, 75 and 98%) and periods of exposure (6, 12, 24 and 48 hours). Values indicate the number of insects per 100 individuals.

<table>
<thead>
<tr>
<th>Test group</th>
<th>Hours of exposure</th>
<th>Relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Young larvae (&lt; 30mg)</td>
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<td></td>
<td>12</td>
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<td></td>
<td>24</td>
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<td>48</td>
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<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Older larvae (&gt; 100mg)</td>
<td></td>
<td>12</td>
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<tr>
<td></td>
<td>24</td>
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<td>48</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pupae</td>
<td></td>
<td>12</td>
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<td></td>
<td>24</td>
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<td>48</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Survival capacity of Tenebrio molitor life stages at 25°C and various combinations of relative humidity (12, 52, 75 and 98%) and periods of exposure (6, 12, 24 and 48 hours). Values indicate the number of insects surviving per 100 individuals.

<table>
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<th>Test group</th>
<th>Hours of exposure</th>
<th>Relative humidity (%)</th>
<th>12</th>
<th>52</th>
<th>75</th>
<th>98</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young larvae (&lt; 30mg)</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td></td>
<td>12</td>
<td>100</td>
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<td>98</td>
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<tr>
<td></td>
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<td>95</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>95</td>
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<tr>
<td>Older larvae (&gt; 100mg)</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td>24</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td></td>
<td>48</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Pupae</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td></td>
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<td>100</td>
<td>100</td>
<td>100</td>
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<td>48</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Adults</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td></td>
<td>12</td>
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<td></td>
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<td></td>
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<td>100</td>
<td>100</td>
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Table 4. Survival capacity of Tenebrio molitor life stages at 35°C and various combinations of relative humidity (12, 52, 75 and 98%) and periods of exposure (6, 12, 24 and 48 hours). Values indicate the number of insects surviving out of 100 individuals.

<table>
<thead>
<tr>
<th>Test group</th>
<th>Hours of exposure</th>
<th>Relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Young larvae (&lt; 30mg)</td>
<td>6</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>51</td>
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<tr>
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<tr>
<td></td>
<td>48</td>
<td>8</td>
</tr>
<tr>
<td>Older larvae (&gt; 100mg)</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>75</td>
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<tr>
<td></td>
<td>48</td>
<td>51</td>
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<tr>
<td>Pupae</td>
<td>6</td>
<td>100</td>
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<tr>
<td></td>
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<td>100</td>
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<td>Adults</td>
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<td>63</td>
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<td></td>
<td>48</td>
<td>25</td>
</tr>
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</table>
Figure 4. The effects of temperature, relative humidity and length of exposure on the survival capacity of various developmental stages of *Tenebrio molitor*. Data points represent the number of insects surviving out of 100 for the larval and adult stages, and the number of pupae out of 100 successfully completing development and emerging as adults for the pupal stage.
p < .0001) and periods of exposure (F = 394.63, df = 3/36, p < .0001) on survival capacity.

The results shown in Figure 4 and Table 3 for 25°C clearly indicate that within the optimum temperature preference of this species (22° - 28°C), as suggested by Mellanby (1932), Cotton (1950) and Howard (1955), relative humidity extremes as low as 12% or as high as 98% had no detrimental effects on the survival capacity of T. molitor. There was 100% survival at the older larval, pupal and adult developmental stages. Only at the young larval stage was there any mortality at extremes of humidity and even in this case it was minimal (2% - 5%). The results also show that length of exposure was not a critical factor at 25°C. In addition, there was no significant overall mortality at extremes of temperature under moisture levels of 52% - 75%. Thus, 25°C represents a nonstressful temperature under all conditions of humidity and 52% - 75% r.h. represent nonstressful moisture conditions at all test temperatures for this species.

At relative humidity extremes of 12% and 98% (Figure 4), there is a significantly higher mortality at extremes of temperature (10° and 35°) and longer periods of exposure (24 and 48 hours). In addition, there was no significant mortality of older larvae and later developmental stages at any of the test temperatures within the 52% - 75% r.h. range. However, at 52% and 75% r.h. the mortality of the young larvae at
temperature extremes was significantly higher than that found for older larvae. The results also indicate that under dry conditions (12% r.h.), low-temperature extremes (10°C) result in a slightly higher mortality rate than do high-temperature (35°C) extremes for the young larval stage of this species. The converse is true for older larvae in that high temperatures cause higher mortality than do low temperatures under both dry and moist conditions.

The data also indicate that the pupal stage of *T. molitor* is most resistant to temperature and relative humidity extremes whereas the young larvae are least resistant (Figure 4, Table 2 through Table 4). Also, the mortality of older larvae at extremes of temperature and long periods of exposure is higher at 98% than at 12% r.h. It can also be seen that in most cases, high-temperature extremes result in a higher mortality rate than do low temperatures at all humidities and all periods of exposure, suggesting that high temperatures appear to be more stressful for this insect than low temperatures.

The significant differences between the various combinations of temperature, relative humidity and periods of exposure are listed in Table 5 and Table 6.

Changes in Larval Temperature Tolerance

The changes in temperature tolerance throughout the larval stage of *T. molitor* are shown in Figure 5. Under optimal
Table 5. Student-t values for the survival capacity of T. molitor life stages at various combinations of temperature and relative humidity

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Test T° (°C)</th>
<th>Between humidities (%)</th>
<th>df</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young larvae</td>
<td>10°</td>
<td>12 - 98</td>
<td>3</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 - 75</td>
<td>3</td>
<td>13.83**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75 - 98</td>
<td>3</td>
<td>11.78**</td>
</tr>
<tr>
<td>Older larvae</td>
<td>10°</td>
<td>12 - 98</td>
<td>3</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 - 75</td>
<td>3</td>
<td>2.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75 - 98</td>
<td>3</td>
<td>2.98</td>
</tr>
<tr>
<td>Pupae</td>
<td>10°</td>
<td>12 - 98</td>
<td>3</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 - 75</td>
<td>3</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75 - 98</td>
<td>3</td>
<td>2.27</td>
</tr>
<tr>
<td>Adults</td>
<td>10°</td>
<td>12 - 98</td>
<td>3</td>
<td>1.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 - 75</td>
<td>3</td>
<td>4.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75 - 98</td>
<td>3</td>
<td>6.67</td>
</tr>
<tr>
<td>Young larvae</td>
<td>35°</td>
<td>12 - 98</td>
<td>3</td>
<td>0.87</td>
</tr>
<tr>
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<td>12 - 75</td>
<td>3</td>
<td>11.85**</td>
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<tr>
<td></td>
<td></td>
<td>75 - 98</td>
<td>3</td>
<td>10.99*</td>
</tr>
<tr>
<td>Older larvae</td>
<td>35°</td>
<td>12 - 98</td>
<td>3</td>
<td>4.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 - 75</td>
<td>3</td>
<td>5.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75 - 98</td>
<td>3</td>
<td>9.22*</td>
</tr>
<tr>
<td>Pupae</td>
<td>35°</td>
<td>12 - 98</td>
<td>3</td>
<td>2.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 - 75</td>
<td>3</td>
<td>2.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75 - 98</td>
<td>3</td>
<td>5.39</td>
</tr>
<tr>
<td>Adults</td>
<td>35°</td>
<td>12 - 98</td>
<td>3</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 - 75</td>
<td>3</td>
<td>6.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75 - 98</td>
<td>3</td>
<td>9.65*</td>
</tr>
</tbody>
</table>

*Significant at p < .05, df = 1.

**Significant at p < .01, df = 1.
Table 6. Student-t values for the survival capacity of T. molitor life stages between temperatures at various relative humidities and periods of exposure

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Humidity (%)</th>
<th>Exposure (Hours)</th>
<th>Between temperatures (°C)</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young larvae</td>
<td>12</td>
<td>6</td>
<td>35 - 25</td>
<td>7.82*</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>48</td>
<td>35 - 25</td>
<td>18.46**</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>48</td>
<td>10 - 25</td>
<td>17.46**</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>6</td>
<td>10 - 25</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>48</td>
<td>35 - 25</td>
<td>9.23*</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>6</td>
<td>35 - 25</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>48</td>
<td>10 - 25</td>
<td>5.01</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>48</td>
<td>35 - 25</td>
<td>11.64*</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>6</td>
<td>35 - 25</td>
<td>5.82</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>48</td>
<td>35 - 25</td>
<td>18.06**</td>
</tr>
<tr>
<td>Older larvae</td>
<td>12</td>
<td>48</td>
<td>10 - 25</td>
<td>5.01</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>48</td>
<td>35 - 25</td>
<td>9.84*</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>48</td>
<td>35 - 25</td>
<td>5.82</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>48</td>
<td>35 - 25</td>
<td>2.20</td>
</tr>
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<td></td>
<td>98</td>
<td>48</td>
<td>10 - 35</td>
<td>4.41</td>
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<tr>
<td></td>
<td>98</td>
<td>48</td>
<td>35 - 25</td>
<td>7.66*</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>48</td>
<td>35 - 25</td>
<td>15.25**</td>
</tr>
<tr>
<td>Pupae</td>
<td>12</td>
<td>48</td>
<td>35 - 25</td>
<td>5.01</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>48</td>
<td>35 - 25</td>
<td>8.83*</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>48</td>
<td>10 - 35</td>
<td>3.54</td>
</tr>
<tr>
<td>Adults</td>
<td>12</td>
<td>48</td>
<td>10 - 25</td>
<td>13.45**</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>48</td>
<td>35 - 25</td>
<td>15.05**</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>48</td>
<td>10 - 25</td>
<td>12.04*</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>48</td>
<td>35 - 25</td>
<td>17.26**</td>
</tr>
</tbody>
</table>

*Significant at p < .10, df = 1.

**Significant at p < .05, df = 1.
Figure 5. The mean survival time in minutes of *Tenebrio molitor* larvae exposed to a lethal temperature of 42 degrees C at relative humidities of 12%, 75% and 98%. Each data point represents the mean value for 10 larvae at each larval instar.
relative humidity conditions (75%), there is an increase in
the amount of time required to produce mortality from 22.5 min.
for the first larval instar to 104 min. for the larval instar
27. At all relative humidities (12, 75 and 98%) there is a
significant progressive increase in the mean survival time from
the first to the last larval instar ($F = 7.71$, $df = 2/26$,
$p < .01$) for this species at an upper lethal temperature of
42°C. Analysis of variance indicates that there is a signifi­
cant difference between the mean survival time of larvae tested
at 12% r.h. and those tested at 98% r.h. ($F = 556.67$, $df =
1/26$, $p < .0001$). However, there is no significant difference
between 12 and 75% and 75 and 98% groups (Table 7). In addi­
tion, the greatest survival time is manifested by older larvae
under extremely dry (12%) conditions (Figure 5).

Table 7. Analysis of variance for changes in larval tempera­
ture tolerance of $T. molitor$ at 42°C and 12, 75 and
98% relative humidity

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>MS$^a$</th>
<th>MS$^b$</th>
<th>$F = \text{MS}^a/\text{MS}^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All groups (12, 75 and 98%)</td>
<td>2/26</td>
<td>85234.80</td>
<td>57850.72</td>
<td>1.47</td>
</tr>
<tr>
<td>Between groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12%/75%</td>
<td>1/26</td>
<td>65258.79</td>
<td>86752.27</td>
<td>0.75</td>
</tr>
<tr>
<td>75%/98%</td>
<td>1/26</td>
<td>166878.35</td>
<td>86757.96</td>
<td>1.92</td>
</tr>
<tr>
<td>12%/98%</td>
<td>1/26</td>
<td>23337.70</td>
<td>41.92</td>
<td>556.67*</td>
</tr>
</tbody>
</table>

$^a$Mean square between groups.

$^b$Mean square within, error term.

*Significant at $p < .0001$. 

Longevity of Adults

The effects of various combinations of temperature and relative humidity on the longevity of *T. molitor* adult males and females are shown in Figure 6. The results indicate that at all test temperatures the adult females manifest longer life spans than the males (Table 8). Figure 6 also shows that the longevity of both males and females is shortest at 35°C regardless of humidity, and the longest life spans for this species are found for the females at 10° and 25°C. In addition, there is a significant difference in the mean longevity between 12% and 75% r.h. and 12% and 98% r.h. for males and females at all test temperatures (Table 9). No difference in longevity was found between 75% and 98% relative humidities. Dry conditions (12%) have the most deleterious effect on longevity at all temperatures (Figure 6). Conversely, the longest life spans were found at 75% r.h. The data also indicate that the mean longevity for males and females is slightly less at 98% r.h. than at 75% r.h.

Oviposition Rate

The effects of temperature and relative humidity on the oviposition rate of *T. molitor* are shown in Figure 7 and Table 10. The data show that the highest oviposition rates were found at temperature of 25° and 35°C, and at relative humidities of 75% and 98% (Figure 7). There were no significant
Figure 6. The mean longevity of adult Tenebrio molitor males and females as a function of temperature and relative humidity. Longevity was expressed as the life span in days after pupal emergence. Each data point represents the mean value for 20 insects.
MEAN LONGEVITY OF ADULTS EXPRESSED AS THE LIFE SPAN IN DAYS AFTER PUPAL EMERGENCE

RELATIVE HUMIDITY (%)

98
75
12

35°C (MALES)
35°C (FEMALES)
10°C (MALES)
25°C (MALES)
25°C (FEMALES)
Table 8. Student-\(t\) values between the longevity of adult *T. molitor* males and females at various temperatures and relative humidities

<table>
<thead>
<tr>
<th>Relative humidity (%)</th>
<th>Test (T^\circ) (°C)</th>
<th>Mean longevity (days)</th>
<th>(t)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>19.55</td>
<td>24.55</td>
</tr>
<tr>
<td>75</td>
<td>10</td>
<td>80.00</td>
<td>96.55</td>
</tr>
<tr>
<td>98</td>
<td>10</td>
<td>60.08</td>
<td>80.25</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>15.00</td>
<td>22.60</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
<td>78.90</td>
<td>94.10</td>
</tr>
<tr>
<td>98</td>
<td>25</td>
<td>72.00</td>
<td>85.70</td>
</tr>
<tr>
<td>12</td>
<td>35</td>
<td>04.75</td>
<td>08.70</td>
</tr>
<tr>
<td>75</td>
<td>35</td>
<td>38.45</td>
<td>43.95</td>
</tr>
<tr>
<td>98</td>
<td>35</td>
<td>29.80</td>
<td>38.10</td>
</tr>
</tbody>
</table>

*Significant at \(p < .01\), df = 19.

**Significant at \(p < .001\), df = 19.

differences between the number of eggs laid per female per 48 hours at these temperatures and humidities (Table 10). At 12% r.h., however, the oviposition rate was significantly lower at 25° and 35°C. There were no eggs deposited by *T. molitor* females at 10°C regardless of the relative humidity (Figure 7). The results also indicate a significant difference between the oviposition rates at 12% and 75% r.h. and 12% and 98% r.h. at temperatures of 25° and 35°C (Table 10). No significant differences were found for the rates of oviposition at 75% and 98% r.h. In addition, there were no significant differences between the number of eggs laid at 25° and 35°C at all conditions of relative humidity (Table 11).
Table 9. Student-t values for the longevity of adult *T. molitor* males and females between relative humidities at various temperatures

<table>
<thead>
<tr>
<th>Test T° (°C)</th>
<th>Sex</th>
<th>Mean longevity (days)</th>
<th>Humidities (%)</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Males</td>
<td>19.55</td>
<td>75</td>
<td>19.11*</td>
</tr>
<tr>
<td>10</td>
<td>Males</td>
<td>19.55</td>
<td>98</td>
<td>18.96*</td>
</tr>
<tr>
<td>10</td>
<td>Females</td>
<td>24.55</td>
<td>75</td>
<td>18.96*</td>
</tr>
<tr>
<td>10</td>
<td>Females</td>
<td>24.55</td>
<td>98</td>
<td>22.51*</td>
</tr>
<tr>
<td>25</td>
<td>Males</td>
<td>15.00</td>
<td>78.90</td>
<td>29.19*</td>
</tr>
<tr>
<td>25</td>
<td>Males</td>
<td>15.00</td>
<td>94.10</td>
<td>22.48*</td>
</tr>
<tr>
<td>25</td>
<td>Females</td>
<td>22.60</td>
<td>94.10</td>
<td>29.39*</td>
</tr>
<tr>
<td>25</td>
<td>Females</td>
<td>22.60</td>
<td>85.75</td>
<td>22.96*</td>
</tr>
<tr>
<td>35</td>
<td>Males</td>
<td>4.75</td>
<td>38.45</td>
<td>16.75*</td>
</tr>
<tr>
<td>35</td>
<td>Males</td>
<td>4.75</td>
<td>38.45</td>
<td>15.55*</td>
</tr>
<tr>
<td>35</td>
<td>Males</td>
<td>38.45</td>
<td>29.80</td>
<td>2.47</td>
</tr>
<tr>
<td>35</td>
<td>Females</td>
<td>8.70</td>
<td>43.95</td>
<td>14.66*</td>
</tr>
<tr>
<td>35</td>
<td>Females</td>
<td>8.70</td>
<td>38.10</td>
<td>19.74*</td>
</tr>
<tr>
<td>35</td>
<td>Females</td>
<td>43.95</td>
<td>38.10</td>
<td>2.15</td>
</tr>
</tbody>
</table>

*Significant at p < .001, df = 19.

Table 10. Student-t values for the oviposition rate of *T. molitor* between relative humidities at various test temperatures

<table>
<thead>
<tr>
<th>Test T° (°C)</th>
<th>Mean oviposition rate/female</th>
<th>Humidities (%)</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>98</td>
</tr>
<tr>
<td>25</td>
<td>24.00</td>
<td>111.00</td>
<td>17.77*</td>
</tr>
<tr>
<td>25</td>
<td>24.00</td>
<td>94.10</td>
<td>15.20*</td>
</tr>
<tr>
<td>35</td>
<td>17.50</td>
<td>100.40</td>
<td>16.96*</td>
</tr>
<tr>
<td>35</td>
<td>17.50</td>
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<td>21.08*</td>
</tr>
<tr>
<td>35</td>
<td>100.40</td>
<td>95.90</td>
<td>0.77</td>
</tr>
</tbody>
</table>

*Significant at p < .001, df = 9.
Figure 7. The mean oviposition rate of Tenebrio molitor as a function of temperature and relative humidity. Oviposition rate was recorded as the number of eggs laid per female over a 48 hour period. Each data point represents the mean number of eggs laid for 10 insects.
Table 11. Student-t values for the oviposition rate of *T. molitor* between 25° and 35°C at various relative humidities

<table>
<thead>
<tr>
<th>Relative humidity (%)</th>
<th>Mean oviposition rate/female</th>
<th>Temperature (°C)</th>
<th>t-valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>25.00</td>
<td>17.50</td>
<td>2.43</td>
</tr>
<tr>
<td>75</td>
<td>111.00</td>
<td>100.40</td>
<td>1.66</td>
</tr>
<tr>
<td>98</td>
<td>94.10</td>
<td>95.90</td>
<td>0.34</td>
</tr>
</tbody>
</table>

a$t$-values not significant.

Locomotor Activity

The preconditioning effects of temperature, relative humidity and period of exposure on the burrowing activity of *T. molitor* larvae are shown in Figure 8. Analysis of variance showed no significant effects of preconditioning at various humidities on burrowing speed at all periods of exposure ($F = 3.25, df = 1/2$). However, the overall effects of conditioning temperature ($F = 2348.91, df = 2/2, p < .0001$) and period of exposure ($F = 451.69, df = 3/181, p < .0001$) on subsequent burrowing speed were highly significant. The most rapid burrowing speeds resulted from preconditioning temperatures of 25° and 35°C. No significant differences in burrowing speeds were found at 25° and 35°C as a function of the period of exposure (Table 12). At 10°C, on the other hand, there was a significant effect of exposure periods on burrowing speeds.
Figure 8. The mean burrowing time in seconds of *Tenebrio molitor* larvae at 25 degrees C as a function of temperature, relative humidity and length of exposure. Each data point represents the mean value for 10 insects.
Table 12. Student-t values for the burrowing speed of *T. molitor* larvae at 25°C between periods of exposure at various preconditioning temperatures and relative humidities

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Relative humidity (%)</th>
<th>Mean burrowing speed (secs)</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Periods of exposure (hrs)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
<td>73.60</td>
<td>76.90</td>
</tr>
<tr>
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</tr>
<tr>
<td>25</td>
<td>75</td>
<td>73.60</td>
<td>76.90</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
<td>73.60</td>
<td>76.90</td>
</tr>
<tr>
<td>35</td>
<td>75</td>
<td>61.70</td>
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</tbody>
</table>

*Significant at p < .01, df = 9.

**Significant at p < .001, df = 9.
At one hour of exposure to 10°C, the larvae were extremely sluggish and burrowing speeds were very slow (Figure 8). At 6 and 12 hours of exposure, burrowing speeds progressively increased to a significant degree over those found at one hour exposure (Table 12), and after 24 hours, the larvae were burrowing almost as rapidly as those that had been conditioned at an optimal temperature of 25°C thereby demonstrating an acclimatory response of burrowing activity to low temperatures.

Burrowing speeds at preconditioning temperatures of 10° and 35°C were significantly different at one (t = 33.26, df = 9, p < .001), six (t = 25.06, df = 9, p < .001), twelve (t = 14.19, df = 9, p < .001) and twenty-four (t = 5.97, df = 9, p < .001) hours of exposure. At 10° and 25°C there were significant differences at one (t = 21.73, df = 9, p < .001), six (t = 18.76, df = 9, p < .001) and twelve (t = 10.04, df = 9, p < .001) hours of exposure. At 24 hours of exposure, however, the burrowing speeds were not significantly different (t = 2.04, df = 9) further substantiating cold-temperature compensation after 24 hours.

The effects of temperature, relative humidity and period of exposure on the running speed of T. molitor adults are shown in Figure 9. Analysis of variance showed no significant effect of preconditioning humidity on running speed at all periods of exposure (F = 5.64, df = 1/2) although running speeds were slightly slower under dry conditions (12%). However, the
Figure 9. The mean running time in seconds of Tenebrio molitor adults as a function of temperature, relative humidity and length of exposure. Each data point represents the mean value for 10 insects.
overall effects of preconditioning temperature ($F = 5403.20$, $df = 2/2$, $p < .001$) and period of exposure ($F = 211.10$, $df = 3/181$, $p < .0001$) on running speed were highly significant. The most rapid ambulatory behavior of adult insects resulted from preconditioning temperatures of $35^\circ C$ (12% and 75% r.h.) and $25^\circ C$, respectively, at all periods of exposure (Figure 9). Only at 24 hours of exposure were significant differences found between running speeds at $25^\circ C$ and $35^\circ C$ ($t = 3.96$, $df = 9$, $p < .01$). No significant differences in running speed were found at $25^\circ C$ and $35^\circ C$ as a function of the period of exposure (Table 14). Running speeds at $10^\circ C$ and $35^\circ C$ were significantly different at one ($t = 21.08$, $df = 9$, $p < .001$), six ($t = 13.86$, $df = 9$, $p < .001$), twelve ($t = 7.09$, $df = 9$, $p < .001$) and twenty-four ($t = 4.03$, $df = 9$, $p < .01$) hours of exposure. Running speeds at $10^\circ C$ and $25^\circ C$ were significantly different at one ($t = 19.62$, $df = 9$, $p < .001$), six ($t = 6.75$, $df = 9$, $p < .001$) and twelve ($t = 4.91$, $df = 9$, $p < .001$) hours of exposure. However, at 24 hours of exposure, the running speeds were almost identical ($t = .46$) indicating some degree of thermal compensation. The data also indicate that at $10^\circ C$ there was a significant effect of exposure period on running speed (Table 13). At one hour of exposure at $10^\circ C$, the adult insects were highly lethargic and locomotor activity was suppressed (Figure 9). Running speeds progressively increased, however, with increasing periods of exposure. After 24 hours,
Table 13. Student-t values for the running speed of *T. molitor* adults at 25°C between periods of exposure at various preconditioning temperatures and relative humidities

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Relative humidity (%)</th>
<th>Mean running speed (secs)</th>
<th>t-value</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Periods of exposure (hrs)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>25</td>
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<td>85.50</td>
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</table>

* Significant at p < .01, df = 9.

** Significant at p < .001, df = 9.
the adults were moving almost as rapidly at 10°C as they did under more optimal temperature conditions (25°C). This exposure effect also demonstrates some degree of temperature compensation.

Dispersion Behavior

The relationship between temperature, moisture and thermal acclimation and their effects on the dispersion of T. molitor larvae are shown in Figure 10 through Figure 15 and Table 14. Analysis of variance showed highly significant vertical layer (F = 23.61, df = 3/48, p < .0001) and temperature/vertical layer interactions (F = 2.98, df = 12.48, p < .003). The larvae were divided into 5 groups based on the 5 temperature conditions of the experiment: (1) Group 1: larvae acclimated (reared) at 25°C and tested at 10°C (25°a/10°t); (2) Group 2: 25°a/25°t; (3) Group 3: 25°a/35°t; (4) Group 4: larvae acclimated at 10°C for 24 hours and then tested at 10°C (10°a/10°t); (5) Group 5: 35°a/35°t. The analysis of variance between these groups is shown in Table 15. Significant differences in vertical dispersion (VL) were found between Groups 1 and 2, 1 and 3, 1 and 4, 1 and 5, 2 and 4, 2 and 5, and 3 and 4. No differences in vertical dispersion were found for the 25° and 35°C groups, and for 25°/35° and 10° acclimated groups.
Figure 10. Dispersion pattern of *Tenebrio molitor* larvae as a function of temperature, thermal acclimation and time of dispersion. Each data point represents the number of larvae out of 50 insects found at each vertical layer. Vertical layers are top, middle-top (MT), middle-bottom (MB) and bottom. The experimental groups were as follows: (1) Group 1: larvae taken from normal rearing conditions (acclimated at 25°C) and tested for dispersion at 10°C (25°a/10°t); (2) Group 2: larvae taken from rearing conditions and tested at 25°C (25°a/25°t); (3) Group 3: larvae taken from rearing conditions and tested at 35°C (25°a/35°t); (4) Group 4: larvae acclimated at 10°C for 24 hours prior to testing at 10°C (10°a/10°t); (5) Group 5: larvae acclimated at 35°C for 24 hours prior to testing at 35°C (35°a/35°t)
Figure 11. Diagrammatic representation of the vertical and horizontal dispersion pattern of *Tenebrio molitor* larvae taken from normal rearing conditions (acclimated at 25°C) and tested for dispersion at 10°C (25°a/10°t). Patterns are shown as a function of the time in hours in the dispersion cube. The number to the right of each tray indicates the total number of insects in that vertical layer.
Figure 12. Diagrammatic representation of the vertical and horizontal dispersion pattern of *Tenebrio molitor* larvae taken from normal rearing conditions (25°C) and tested for dispersion at 25°C (25°a/25°t). Patterns are shown as a function of the time in hours in the dispersion cube.
Figure 13. Diagrammatic representation of the vertical and horizontal dispersion pattern of *Tenebrio molitor* larvae taken from normal rearing conditions (acclimated at 25°C) and tested for dispersion at 35°C (25°a/35°t). Patterns are shown as a function of the time in hours in the dispersion cube.
TIME (HOURS)

T

MT

MB

B

.5

2

17

10

10

21

2

9

10

17

4

3

10

16

14

21
Figure 14. Diagrammatic representation of the vertical and horizontal dispersion pattern of *Tenebrio molitor* larvae acclimated at 10°C for 24 hours prior to testing for dispersion at 10°C (10°a/10°t). Patterns are shown as a function of time in hours in the dispersion cube.
Figure 15. Diagrammatic representation of the vertical and horizontal dispersion pattern of *Tenebrio molitor* larvae acclimated at $35^\circ$C for 24 hours prior to testing for dispersion at $35^\circ$C ($35^\circ\text{a}/35^\circ\text{t}$). Patterns are shown as a function of the time in hours in the dispersion cube.
Table 14. The number of *T. molitor* larvae in the top (T), middle top (MT), middle bottom (MB) and bottom (B) vertical layers of the dispersion cube at various acclimation (a) and test (t) temperatures and dispersion times

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<th>2</th>
<th>3</th>
<th>4</th>
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<td>25°a/10°t</td>
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<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>21</td>
<td>24</td>
<td>21</td>
<td>17</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MB</td>
<td>28</td>
<td>20</td>
<td>27</td>
<td>32</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>25°a/25°t</td>
<td>T</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>13</td>
<td>16</td>
<td>9</td>
<td>3</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MB</td>
<td>21</td>
<td>18</td>
<td>16</td>
<td>9</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>11</td>
<td>13</td>
<td>25</td>
<td>38</td>
<td>15</td>
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</tr>
<tr>
<td>25°a/35°t</td>
<td>T</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>17</td>
<td>6</td>
<td>10</td>
<td>27</td>
<td>10</td>
<td></td>
</tr>
<tr>
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<td>MB</td>
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<td>13</td>
<td>17</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>10</td>
<td>31</td>
<td>14</td>
<td>5</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>10°a/10°t</td>
<td>T</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>6</td>
<td>1</td>
<td>13</td>
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<tr>
<td></td>
<td>MB</td>
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<td>17</td>
<td>17</td>
<td>14</td>
<td>29</td>
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</tr>
<tr>
<td></td>
<td>B</td>
<td>8</td>
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<td>27</td>
<td>35</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>35°a/35°t</td>
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<td>8</td>
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<td>7</td>
<td></td>
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<td>23</td>
<td>19</td>
<td>19</td>
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<tr>
<td></td>
<td>B</td>
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<td>0</td>
<td>11</td>
<td>6</td>
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</table>
Table 15. Analysis of variance for the vertical dispersion of *T. molitor* larvae between the 5 temperature groups. (a = acclimation temperature; t = test temperature; VL = vertical layer)

<table>
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<tr>
<th>Temperature groups (°C)</th>
<th>d.f.</th>
<th>MS(^a)</th>
<th>MS(^b)</th>
<th>(F = \text{MS}^a/\text{MS}^b)</th>
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<td>476.13</td>
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<td>13.52*</td>
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<td>35.21</td>
<td>1.00</td>
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<td>21.28**</td>
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<td>(VL)</td>
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<td>323.13</td>
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<td>8.58*</td>
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<td>37.63</td>
<td>0.97</td>
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<td>49.08</td>
<td>1.26</td>
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<td>1113.66</td>
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<td>0.26</td>
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<td>(VL) x Time</td>
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<td>96.58</td>
<td>0.27</td>
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<tr>
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<td>3/12</td>
<td>555.53</td>
<td>13.80</td>
<td>40.25**</td>
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<tr>
<td>(VL)</td>
<td></td>
<td>13.13</td>
<td>13.80</td>
<td>0.95</td>
</tr>
<tr>
<td>(VL) x Time</td>
<td></td>
<td>146.53</td>
<td>13.80</td>
<td>10.61*</td>
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<tr>
<td>Condition</td>
<td>(VL)</td>
<td>(VL) x Temperature</td>
<td>(VL) x Time</td>
<td>Mean Square</td>
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<td>----------------------------</td>
<td>--------</td>
<td>--------------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>25°a/25°t and 35°a/35°t</td>
<td>3/12</td>
<td>431.13</td>
<td>47.95</td>
<td>8.99*</td>
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<td></td>
</tr>
<tr>
<td>25°a/35°t and 10°a/10°t</td>
<td>3/12</td>
<td>434.86</td>
<td>125.00</td>
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<td></td>
</tr>
<tr>
<td>10°a/10°t and 35°a/35°t</td>
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<td>430.06</td>
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<td>5.45</td>
</tr>
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</tbody>
</table>

*Mean square between groups.

bMean square within, error term.

*Significant at p < .001.

**Significant at p < .0001.
The results shown in Figure 10 and Table 14 indicate that under optimal conditions (25°a/25°t) there is an initial downward dispersion of the larvae with 38 of the 50 larvae being found in the bottom vertical layer after 3 hours. The larvae also exhibit a strong reluctance toward movement into the top vertical layer (Figure 12). Under cold-temperature conditions (25°a/10°t) the larvae remain largely in the middle vertical layers with very few insects being found in the top and bottom layers at any time (Figure 11). However, after a 24-hour acclimation period at 10°C, prior to testing at 10°C (10°a/10°t), the larvae exhibit a dispersion pattern (Figure 14) very similar to that recorded for these insects under more optimal and normal conditions (25°a/25°t). Larvae in the 25°a/35°t group also exhibited an initial downward dispersion (Figure 13) into the bottom layer; in this case, however, the movement was more rapid at this higher test temperature (Figure 10) with 31 of the 50 larvae being found in the bottom vertical layer after only one hour in the cube. The dispersion pattern recorded for the 35°a/35°t group (Figure 15) is an extremely erratic one characterized by movement of the larvae into and out of the middle-top, middle-bottom and bottom layers and only minimal dispersion into the top vertical layer.

Moisture content determinations of the grain under these various test temperature conditions were performed. Within the temperature range of 10° to 35°C, the relative humidity value
within the constant temperature cabinets was 75% when a large tray of water was inserted at the bottom of the cabinet. The moisture content of the grain under these conditions of temperature and humidity are shown in Figure 16.

The relationship between temperature, moisture and thermal acclimation and their effects on the dispersion of *T. molitor* adults are shown in Figure 17 through Figure 22 and Table 16. Analysis of variance showed highly significant vertical layer \((F = 271.67, \text{df} = 3/48, p < .0001)\) and temperature/vertical layer \((F = 47.96, \text{df} = 12/48, p < .0001)\) interactions. The adults were divided into the same temperature groups as the larvae (Table 18): (1) Group 1: 25°a/10°t; (2) Group 2: 25°a/25°t; Group 3: 25°a/35°t; (4) Group 4: 10°a/10°t; (5) Group 5: 35°a/35°t. The analysis of variance between these groups is shown in Table 17. Significant differences in vertical dispersion (VL) were found between all groups. Significant temperature effects on vertical dispersion were found between Groups 1 and 2, 1 and 3, 1 and 4, 1 and 5, 2 and 4, and 3 and 4. The effects of dispersion time were not significant for any of the groups.

The results shown in Figure 17, Figure 19 and Table 16 indicate that under optimal temperatures (25°C) the adults of this species exhibit a strong inclination to move to and remain in the upper vertical layer of the cube. Under cold-temperature conditions (25°a/10°t) the adults remain largely in the middle vertical layers and away from the periphery of the cube (Figure
Figure 16. The percent moisture content of the grain as a function of temperature and moisture
TIME

PERCENT CONTENT OF GRAIN

20

15

10

5

10°C / 75%

25°C / 75%

35°C / 75%

HOURS
Table 16. The number of *T. molitor* adults in the top (T), middle top (MT), middle bottom (MB) and bottom (B) vertical layers of the dispersion cube at various acclimation (a) and test (t) temperatures and dispersion times

<table>
<thead>
<tr>
<th>Temperature condition (°C)</th>
<th>Vertical layer</th>
<th>Number of adults/vertical layer</th>
<th>Time (hrs)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>.5</td>
</tr>
<tr>
<td>25°a/10°t</td>
<td></td>
<td>T</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MT</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MB</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>2</td>
</tr>
<tr>
<td>25°a/25°t</td>
<td></td>
<td>T</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MT</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MB</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>0</td>
</tr>
<tr>
<td>25°a/35°t</td>
<td></td>
<td>T</td>
<td>37</td>
</tr>
<tr>
<td></td>
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<td>MT</td>
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<td>MB</td>
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<td>2</td>
</tr>
<tr>
<td>10°a/10°t</td>
<td></td>
<td>T</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MT</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
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<td>35°a/35°t</td>
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</tr>
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<td></td>
<td></td>
<td>MT</td>
<td>11</td>
</tr>
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<td></td>
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<tr>
<td></td>
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<td>B</td>
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</tbody>
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Table 17. Analysis of variance for the vertical dispersion of T. molitor adults between the 5 temperature groups. (a = acclimation temperature; t = test temperature; VL = vertical layer)

<table>
<thead>
<tr>
<th>Temperature groups (°C)</th>
<th>d.f.</th>
<th>MS^a</th>
<th>MS^b</th>
<th>F = MS^a/MS^b</th>
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<tr>
<td>(VL)</td>
<td>3/12</td>
<td>730.86</td>
<td>12.58</td>
<td>58.08**</td>
</tr>
<tr>
<td>(VL) x Temperature</td>
<td>3/12</td>
<td>1785.00</td>
<td>12.58</td>
<td>141.85**</td>
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<tr>
<td>(VL) x Time</td>
<td>12/12</td>
<td>9.45</td>
<td>12.58</td>
<td>0.75</td>
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<tr>
<td>25°a/10°t and 25°a/35°t</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(VL)</td>
<td>3/12</td>
<td>717.53</td>
<td>6.03</td>
<td>118.92**</td>
</tr>
<tr>
<td>(VL) x Temperature</td>
<td>3/12</td>
<td>1619.53</td>
<td>6.03</td>
<td>268.43**</td>
</tr>
<tr>
<td>(VL) x Time</td>
<td>12/12</td>
<td>17.36</td>
<td>6.03</td>
<td>2.87</td>
</tr>
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<td>25°a/10°t and 10°a/10°t</td>
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<tr>
<td>(VL)</td>
<td>3/12</td>
<td>774.06</td>
<td>12.28</td>
<td>63.01**</td>
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<td>(VL) x Temperature</td>
<td>3/12</td>
<td>994.20</td>
<td>12.28</td>
<td>76.86**</td>
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<td>(VL) x Time</td>
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<td>16.65</td>
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<td>(VL)</td>
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<td>103.52**</td>
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<td>(VL) x Time</td>
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<td>13.06</td>
<td>1.51</td>
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<td>10°a/10°t and 35°a/35°t</td>
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<td>(VL)</td>
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<tr>
<td>(VL) x Temperature</td>
<td>3/12</td>
<td>95.66</td>
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<td>(VL) x Time</td>
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<td>3/12</td>
</tr>
<tr>
<td>(VL) x Time</td>
<td>12/12</td>
</tr>
</tbody>
</table>

a Mean square between groups.
b Mean square within, error term.

* Significant at p < .001.
** Significant at p < .001.
Figure 17. Dispersion pattern of Tenebrio molitor adults as a function of temperature, thermal acclimation and time of dispersion. Each data point represents the number of adults out of 50 insects found at each vertical layer. Vertical layers are top, middle-top (MT), middle-bottom (MB), and bottom. The experimental groups were as follows: (1) Group 1: adults taken from normal rearing conditions (acclimated at 25°C) and tested for dispersion at 10°C (25°a/10°t); (2) Group 2: adults taken from rearing conditions and tested at 25°C (25°a/25°t); (3) Group 3: adults taken from rearing conditions and tested at 35°C (25°a/35°t); (4) Group 4: adults acclimated at 10°C for 24 hours prior to testing at 10°C (10°a/10°t); (5) Group 5: adults acclimated at 35°C for 24 hours prior to testing at 35°C (35°a/35°t)
Figure 18. Diagrammatic representation of the vertical and horizontal dispersion pattern of *Tenebrio molitor* adults taken from normal rearing conditions (acclimated at 25°C) and tested for dispersion at 10°C (25°a/10°t). Patterns are shown as a function of the time in hours in the dispersion cube.
Figure 19. Diagrammatic representation of the vertical and horizontal dispersion pattern of *Tenebrio molitor* adults taken from normal rearing conditions (acclimated at 25°C) and tested for dispersion at 25°C (25°a/25°t). Patterns are shown as a function of the time in hours in the dispersion cube.
18). After a 24-hour acclimation period at 10°C, however, the adults exhibit a dispersion pattern (Figure 17 and Figure 21) similar to that recorded for these insects under more optimal conditions (25°a/25°t) in that most of the beetles are in the upper vertical layer. Adults in the 25°a/35°t group also exhibited a strong inclination to remain in the upper vertical layer (Figure 17 and Figure 20) and there was no significant vertical layer/temperature difference between this group and the 25°a/25°t group (Figure 22). Under all conditions, the adults appear to be markedly top-dwellers.
Figure 20. Diagrammatic representation of the vertical and horizontal dispersion pattern of *Tenebrio molitor* adults taken from normal rearing conditions (acclimated at 25°C) and tested for dispersion at 35°C (25°a/35°t). Patterns are shown as a function of time in hours in the dispersion cube.
Figure 21. Diagrammatic representation of the vertical and horizontal dispersion pattern of *Tenebrio molitor* adults acclimated at 10°C for 24 hours prior to testing for dispersion at 10°C (10°a/10°t). Patterns are shown as a function of the time in hours in the dispersion cube.
Figure 22. Diagrammatic representation of the vertical and horizontal dispersion pattern of Tenebrio molitor adults acclimated at 35°C for 24 hours prior to testing for dispersion at 35°C (35°a/35°t). Patterns are shown as a function of the time in hours in the dispersion cube.
DISCUSSION

Survival Capacity

One of the important generalizations to keep in mind when studying the effects of physical limiting factors such as temperature and moisture on the biology of organisms is the consideration that physical environmental factors rarely operate independently of one another. Temperature and moisture are so generally important in terrestrial habitats and are so closely interacting that they are generally conceded to be among the most important climatic factors present in the environment (Almquist, 1970; Andrewartha and Birch, 1954; Bacot and Martin, 1924; Bailey, 1969; Bursell, 1964b; Davidson and Andrewartha, 1948; Edney, 1957; Seymour, 1974; Thomson, 1938; Uvarov, 1931). As is true of other organisms, water constitutes a large proportion of insect tissues, and their survival depends on their ability to conserve water and minimize evaporative water loss. Because of their small size, insects have a low volume to surface area ratio, and, therefore, risk losing an already limited supply of tissue water. For these reasons, both temperature and relative humidity play important roles as limiting factors by influencing the survival capacity of insects. Although earlier investigators suggested that optimum temperature and moisture conditions for *T. molitor* were between 22°-28°C and 70%-80% r.h.,
respectively, these parameters were vaguely defined and stringent humidity controls were lacking. In the present study on the effects of temperature and moisture on the survival capacity of this species, a broad range of temperatures and relative humidities were utilized. In addition, the use of nonvolatile saturated salt solutions as prescribed by recent investigators (Grahm, 1965; Richardson and Malthus, 1955; Winston and Bates, 1960) ensured that accurate humidity controls were present.

The results shown in Figure 3 indicate the relationship between temperature and moisture on the survival capacity of T. molitor eggs measured as a function of their water uptake during embryonic development. Under relatively moist conditions (75% r.h.), successful embryonic development took place at 25°C and 35°C, the rate of development progressing more rapidly at 35°C. In the development of T. molitor eggs at 25°C, there is an initial period of more rapid water absorption (1-4 days) followed by a period of reduced water uptake (5-7 days). Similar findings have been reported for the eggs of Gryllus commodus (Johnson, 1942), the acridid, Austroicetes cruciata (Andrewartha and Birch, 1954), the hemipterans, Corixa punctata (Beament, 1954) and Rhodnius prolixus (Wigglesworth, 1965). These changes in water content during embryonic development reflect the overall physiological changes which are taking place within the egg (Agrell, 1964).
The importance of such water absorption during the development of arthropod eggs has been documented frequently in the literature (Bursell, 1964b; Chapman, 1971; Cloudsley-Thompson, 1970; Kirchner, 1973; Wigglesworth, 1965). Investigations on the morphology of arthropod eggs have indicated that there are specialized regions of the chorion through which most water absorption takes place; these specialized regions varying from one species to another (Bursell, 1964b).

Figure 3 also shows that a temperature of 10°C, even at 75% r.h., is low enough to retard embryonic development completely and water absorption is at a minimum. Similar cold-temperature effects have been reported for the eggs of Melanoplus bivittatus (Salt, 1936), Pteronarcys sp. (Edney, 1954b), Phyllopertha horticola (Wigglesworth, 1965), and Melanoplus differentialis (Agrell, 1964) as well as several species of woodlice (Edney, 1954a) and arachnids (Kirchner, 1973).

Under relatively dry conditions (12% r.h.), the eggs of T. molitor transpired water and lost weight at all temperatures until eventual desiccation took place after 11-15 days (Figure 3). It is generally conceded among investigators that evaporative water loss under dry conditions takes place across the chorion (Bateman, 1967; Beament, 1954; Bodenheimer and Schenkin, 1928; Bursell, 1964a; Buxton, 1932; Chapman, 1971). The chorion of arthropod eggs is formed mainly from the
epithelial follicular cells of the ovariole and differs principally from the integument of later developmental stages in that it is nonchitinous (Blower, 1951; Wigglesworth, 1965). During embryonic development and following formation of the vitelline membrane, the secondary egg membranes (serosal cuticle) are formed which consist of an outer, tanned lipoprotein layer and an inner chitinious layer. Conditions of high temperature or low humidity affect the physical and chemical construct of the chorion resulting in the diffusion of water out of the egg. Due to the small size of arthropod eggs, the degree of water loss that can be tolerated is low. Thus, the necessity for efficient mechanisms for water conservation is clearly evident. Beament (1954) found that the entire chorion of the eggs of Rhodnius prolixus was permeable to water and that eventual resistance to water loss is the result of a primary waxy layer which is laid down on the inside of the chorion (Edney, 1967). Later in development, the serosa produces a secondary waxy layer among the secondary egg membranes. These waxy layers have been verified for numerous insect species (Agrell, 1964; Baldwin and House, 1954; Beament, 1954; Chapman, 1971; Cloudsley-Thompson, 1962b; Coombs and Woodroffe, 1962; Davies, 1928; Eder, 1940; Edney, 1957; Goonewardene and Townshend, 1974; Heber et al., 1973; House et al., 1958; Maloeuf, 1938; Waloff, 1941; Watters, 1966). These waxy layers exhibit similar transpiration/
temperature curves to those of the epicuticle (Edney, 1967). The temperature at which these waxy layers break down has been reported for several species. It is 51.5°C for *Rhodnius prolixus*, 45°C for *Melanoplus bivittatus*, and 39.5°C for *Gryllus commodus* (Johnson, 1942). In the present study, the combination of high stressful temperature (35°C) and dry air (12% r.h.) resulted in the transpiration of water from the eggs of *T. molitor* resulting in a progressive loss of weight and eventual desiccation (Figure 3). In fact, desiccation resulted at 12% r.h. regardless of whether the temperature was high (35°C), optimal (25°C) or low (10°C). Thus, the eggs of this species cannot tolerate dry-air conditions which is generally true for all arthropods studied (Beament, 1954; Bursell, 1964b; Edney, 1957; Flemister, 1964; Wigglesworth, 1965). This is exemplified by the fact that the adults of *T. molitor* generally prefer moist habitats and deposit their eggs in these areas (Cotton, 1927, 1950; Cotton and George, 1929; Dillon and Dillon, 1961; Howard, 1955; Scholz, 1925), thereby ensuring a higher survival capacity for the species.

The results shown in Figure 4 and in Table 2 through Table 4 indicate the relationship between temperature and moisture and their effects on the survival capacity of the larval, pupal and adult stages of *T. molitor*. The results show that at 25°C, which is within the preferred temperature range of this species (22°-28°C), high and low relative
humidity extremes had no detrimental effects on the survival of this insect at all developmental stages. At this temperature, the length of exposure is also not a critical factor. These results confirm previous reports on the preferred temperature of T. molitor and suggest that 25°C represents an optimal temperature for this species.

At 10° and 35°C, however, the interacting effects of temperature, moisture and length of exposure are clearly evident. At these two temperature extremes, relative humidity values in the middle range (52% and 75%) were not as severely limiting as relative humidity extremes of 12% and 98%. This is an excellent example of factor interaction (Odum, 1971) which states that temperature will exert a more severe limiting effect when relative humidity conditions are extreme, and, conversely, relative humidity will exert a more critical role at extremes of temperature.

The higher mortality exhibited by the young larvae at 35°C reflects the fact that due to their smaller size, they cannot tolerate evaporative water loss which results from high temperatures because of their relatively low tissue water content. Also, because of their large surface area to volume ratio, the effects of evaporative water loss are magnified.

The survival capacity of the older larvae at 35°C was more severely reduced under very moist conditions (98% r.h.) than under dry conditions (12% r.h.). This is due to the fact
that larger insects can effectively counteract high ambient temperatures by the process of evaporative cooling. The evaporative loss of water through the integument results in a cooling effect (lowering of body temperature) because the latent heat of vaporization is withdrawn from the body (Clarke, 1967; Edney, 1957; Mellanby, 1954a). Under high environmental moisture conditions, however, evaporative water loss is negated and the limiting effects of high ambient temperatures cannot be as successfully counteracted.

It is well-established that for each insect species there is a fairly well-defined range of temperatures within which the organism remains viable (Bursell, 1964a; Cloudsley-Thompson, 1964, 1970; Mellanby, 1932). The values of this range depend on whether the environmental condition is one of dry heat or humid heat. The exact causes of death at the limits of the viable temperature range are still the subject of much debate and conjecture. However, most investigators maintain that death at low temperatures is attributable to the destructive formation of ice crystals within the tissues (Salt, 1969) and at high temperatures to the denaturation of proteins (Cloudsley-Thompson, 1971; Mellanby, 1954a; Precht, 1968, Prosser, 1961; Read, 1967; Roberts, 1966; Stadtman, 1968; Ushakov, 1964; Zondag, 1963) and breakdown of cellular lipids (Bursell, 1964a; Wigglesworth, 1965). In addition, the general interference with metabolic processes caused by both
high and low temperature extremes has been implicated as well (Chapman, 1971; Prosser, 1961; Tribe and Bowler, 1968; Uvarov, 1931). The upper lethal temperature of T. molitor larvae and adults has been established at 42°-44°C (Altman and Dittmer, 1973) for an exposure period of 24 hours. The chill-coma temperature for this species has been established at 7°-8°C for a similar exposure period (Mutchnor and Richards, 1961). Although upper lethal and chill-coma temperatures were not employed in the present study, temperatures of 35° and 10°C clearly constitute conditions of stress for this species, especially at low and high humidities and at 24-48 hours of exposure (Figure 4).

With respect to humidity effects, the concept of a viable range as described for temperature does not apply (Bursell, 1964b; Flemister, 1964). Humidity affects the survival capacity of arthropods basically through an influence on their water content (Bursell, 1964b; Cloudsley-Thompson, 1959, 1964). The integument of arthropods is not completely impermeable to water. Thus, when an insect is surrounded by an unsaturated atmosphere a vapor pressure gradient is established between the surface of the insect and the ambient air (Clarke, 1967). As a result, water will evaporate from the surface of the insect along this gradient into the general atmosphere. Under these conditions, the rate of evaporation is dependent upon the degree to which the evaporating surface (integument) is
permeable to water and the temperature of the evaporating surface (Alexander and Ewer, 1958; Almquist, 1970; Amos, 1968; Arlian and Wharton, 1974; Cloudsley-Thompson, 1970; Flemister, 1964). Due to the small size of insects, the surface area through which evaporative water loss can take place is large in relation to the quantity of bodily water that they contain. For insects which inhabit hot-dry environments, such as xeric and often stored-product species, desiccation is an important cause of death (Cloudsley-Thompson, 1970). At 24 and 48 hours of exposure, and at a temperature of 35°C, the mortality of T. molitor larvae and adults was significant. This suggests that T. molitor cannot tolerate dry conditions for long periods of time and correlates well with the fact that this species is normally found in moist habitats (Dodds and Ewer, 1952).

Another source of water loss in insects is the loss of water through excretion (Chapman, 1971). In most terrestrial insects, where the need to conserve water is at a premium, nitrogenous wastes are excreted mainly in the form of uric acid. Uric acid is only slightly soluble in water so that water may be separated from it with relatively little osmotic work (Wigglesworth, 1965). Some insect species possess specialized morphological adaptations which affect the re-absorption of water from the feces in the hindgut and rectal regions (Bursell, 1964a). In these species, when the contents
of the midgut are passed into the hindgut they are quite fluid, but become progressively drier as they proceed through the rectum. The highest degree of water reabsorption takes place in the rectum through specialized structures referred to as rectal pads (Chapman, 1971; Maloeuf, 1938). The adults of T. molitor have well-developed rectal pads (Patton and Craig, 1939; Wigglesworth, 1965) for water absorption. Despite this water conservation mechanism, 12% r.h. has a severe limiting effect on this species (Figure 4).

Changes in Larval Temperature Tolerance

The results shown in Figure 5 clearly indicate that there is a progressively increasing resistance to lethal temperatures during larval development at all humidities, although this effect was most dramatic at 12% r.h. Under dry conditions (12% r.h.), 18-27 instar larvae were able to withstand a lethal temperature for a longer period of time than were comparably developed larvae under moist conditions (75% and 98%). This again demonstrates the fact that older and larger larvae can counteract the detrimental effects of high ambient temperatures to some degree by transpiring water (evaporative cooling) which is only possible under dry atmospheric conditions. Younger, and therefore smaller larvae, on the other hand, cannot afford to lose an already limited supply of body water, and as a result dry-air conditions are more lethal to younger
developmental stages (instars 1-16).

Previous investigations concerning changes in temperature tolerance as a function of development in insects have been largely confined to the pupal (Mellanby, 1954b) and adult (Agrell, 1964; Bowler, 1967; Davison, 1969; Hollingsworth and Bowler, 1966) stages. It has been shown for *Dahlbominus fuscipennis* (Baldwin and House, 1954; Baldwin and Riordan, 1956), *Drosophila subobscura* (Hollingsworth and Bowler, 1966), *Glossina morsitans* (Edney, 1967), *Calliphora erythrocephala* (Davison, 1969), *Musca vetustissima* (Cloudsley-Thompson, 1964) and *Tenebrio molitor* (Bowler, 1967) that there is a characteristically drastic loss in temperature tolerance in the first 1-5 days following adult eclosion, and a progressive increase in temperature tolerance during the pupal stage (Mellanby, 1954b). A progressive and rapid loss of resistance to cold temperatures was found for teneral *Glossina*. At 3 days after pupal emergence, for example, 42% of the adults tested survived exposure to 2.5°C for 30 minutes whereas only 30% of 10-day old adults survived this exposure for 10 minutes (Edney, 1967). In the chalcid, *D. fuscipennis*, there is a similar fall in high temperature tolerance from 210 minutes at 42°C for one-day old adults to 60 minutes at 5 days of age (Baldwin and Riordan, 1956). Bowler (1967) reported a sharp loss in temperature tolerance for *T. molitor* adults in the first few days following eclosion. On the first day of adult life the
mean survival time at a lethal temperature of 42°C was 190 minutes under dry-air conditions. This fell to a mean survival time of only 125 minutes after 3 days of age. Conversely, Mellanby (1954b) found a progressive increase in resistance to high lethal temperatures (42°-44°C) during the pupal stage of this species; from 160 minutes on the second day of pupation to 175 minutes on day 6. The results shown in Figure 5 for T. molitor larvae complete the data for this species. Under all humidity conditions, there is a progressive increase in high temperature tolerance during larval development. Similar results have been reported for Tribolium confusum larvae (Davison, 1969). Thus, the overall pattern for temperature tolerance changes during insect development appears to be one of progressively increasing resistance during the larval and pupal stages and sudden decreasing tolerance in the adult stage. The high degree of temperature tolerance manifested by the pupal stage correlates well with the results shown in Figure 4 which indicate the pupal stage of T. molitor to be the most resistant to temperature and relative humidity extremes. This greater pupal resistance to extremes of temperature and relative humidity has obvious survival value to a nonmotile developmental stage. Larvae and adults are able to enact locomotor responses in order to remove themselves from unfavorable environmental conditions; the pupae cannot.
The changes in temperature tolerance recorded for T. molitor larvae reflect the overall physiological changes which are taking place during maturation. The fact that changes in physiological performance as well as the processes of temperature acclimation are paralleled by changes in enzymatic activity and in the induction of isozymes (Agrell, 1964; Chapman, 1971; Fry, 1967; Hochachka, 1971; Precht, 1958, 1968; Prosser, 1961; Rao, 1967) strongly suggest an enzymatic basis for the observed developmental changes in resistance to lethal temperatures.

Longevity of Adults

The life span for an insect species can be obtained by recording the maximal duration of life for the oldest individuals within a population or by taking the mean longevity expressed in days after oviposition, or, as in the case of adults, days after pupal emergence (Allee et al., 1949; Odum, 1971). Although mortality records are known for relatively few insect species (Amos and Morley, 1971), those that are available indicate that each species has a characteristic life span varies widely from one species to another. It is also important to keep in mind that the life span of insects under laboratory conditions is probably different from that found in the normal habitat of the species (Allee et al., 1949; Amos, 1968). Previous investigations on the life span of adult
insects have confirmed the wide variations between species and also that there are differences as a function of sex (Park and Frank, 1948). For example, the mean adult life span (in days) for *Musca domestica* males is 17.5, and 29.0 for females (Wigglesworth, 1965); for *Calliphora erythrocephala* it is 35 (males) and 24 (females); for *Bombyx mori* (Davidson and Andrewartha, 1948) it is 15 (males) and 14 (females); for *Tropia luna* (Belehradek, 1957) it is 6 days for both sexes; it is 13 (males) and 16 (females) for *Ostrinia nubilalis* (Davison, 1971); for *Periplaneta americana* (Nicholson, 1933) it is 200 (males) and 235 (females). Studies on the longevity of *Tribolium confusum* and *T. castaneum* (Gray, 1948; Howe, 1960; Park and Frank, 1948) show that the mean adult life span for *T. confusum* is 56 days at 25°C and 34 days at 34°C; for *T. castaneum* it is 46 days at 25°C and 26 days at 34°C. These results indicate a difference in longevity as a function of sex and temperature; the longer life span occurring at lower temperatures. The results obtained for *T. molitor* (Figure 6) in the present study indicate that the longevity of adult females is greater than that of the males regardless of temperature or relative humidity. There is obvious survival value in having the egg-laying sex live longer. Females of this species require only a single mating and are capable of storing viable sperm throughout their adult life (Howard, 1955). As a result, the longer life span affords the females
a longer period of time in which to deposit fertilized eggs and thus enhance the fecundity of the species.

It is well known that the rate of metabolism of ectothermic organisms is markedly affected by the environmental temperature (Bullock, 1955; Cloudsley-Thompson, 1970; Colhoun, 1954; Edwards and Nutting, 1950; Fry, 1958; Giese, 1968; Gunn, 1942; Hazel and Prosser, 1970; Herter, 1953; Hochachka, 1967; Kanungo and Prosser, 1960; Marzusch, 1952a, 1952b; McFarlane and McLusky, 1972; McWhinnie, 1967; Meats, 1973; Mutchmor and Richards, 1961; Newell, 1966, 1973; Pandey, 1972; Prosser, 1961; Richards, 1956, 1963, 1964; Somero, 1969b). Within the viable temperature range of a species, the higher the temperature the higher the rate of heat production and oxygen consumption. In addition, it has been shown that metabolic rate is inversely proportional to life span (Belehradek, 1957; Mueller and Stern, 1973; Nayar, 1972; Nuttall, 1970; Wigglesworth, 1965). That is, aging is faster for insects subjected to higher environmental temperatures (Rockstein, 1964) which is presumably due to the more rapid accumulation of deleterious substances or greater metabolic stress (energy expenditure) which are characteristic of higher temperature regimes. The data in Figure 6 clearly show this to be the case for T. molitor adults. The adult longevity of both sexes was significantly shorter at 35°C than at 10° or 25°C. In addition, the adult life span of this species is severely reduced under
dry-air conditions (12% r.h.) at all test temperatures. Studies on the effects of humidity on insect longevity are scarce. It was found for *M. domestica* that at lower temperatures the life span was prolonged when humidity was lowered from 80% to 30% (Cloudsley-Thompson, 1964; Flemister, 1964). Studies on *Drosophila melanogaster* (1964) indicated that within a temperature range of 27°-32°C, adult longevity increased with increasing humidity from 40%-90%. However, none of these previous studies employed humidities as low as 12%. It is clear that such extremely dry conditions significantly reduce the adult life span of both sexes of *T. molitor* (Table 9) even at an optimal temperature of 25°C. Also, cold temperatures (10°C) similarly reduce the life span of this species from the values manifested under more optimal conditions (25°C) suggesting that 10°C constitutes low-temperature stress for this species. This correlates well with the deleterious effects reported for 10°C on the survival capacity of *T. molitor* (Figure 4).

With respect to differences in longevity recorded between the two sexes of *T. molitor* (Table 8, Figure 6) at all temperatures and humidities, it is generally stated in the literature that female insects exhibit a longer life span than males of the same species, although there are exceptions (Amos and Morley, 1971; Bailey, 1969; Decker and Andre, 1936; Gray, 1948; Park and Frank, 1948; Robinson, 1926). It should be
emphasized, however, that a comparison of adult life span as a function of sex is difficult due to differences in nutrition (Chapman, 1971), behavior (Cloudsley-Thompson, 1962b), body size and structure (Wigglesworth, 1965). It has been postulated that sexual differences in longevity are due to differences in the quantity of chromosomes (Amos and Morley, 1971; Park and Frank, 1948; Rockstein, 1964) and in the rates of metabolism (Colhoun, 1954; Edwards, 1946; McWhinnie, 1967). In most orders of insects the male is heterogametic and the female homogametic. Thus, some have postulated that the difference in life span between the sexes is due to the presence of subvital recessive genes and as such the heterogametic sex (male) would be more vulnerable than the homogametic sex (Agrell, 1964). One would therefore expect females to live longer than males. This was found to be true for T. molitor in the present study (Figure 6). With respect to metabolic rate, it is well known that the males of most animal species appear to have a higher rate of metabolism than females (Gunn, 1942; Herter, 1953; Marzusch, 1952b; Precht, 1968). Previous investigations have verified higher rates of oxygen consumption in males for several insect species including M. domestica (Edwards and Nutting, 1950), and several species of Lepidoptera (Wigglesworth, 1965). Metabolic differences between the sexes with respect to various tissues have also been reported. Muscle tissue from the legs of P. americana
showed higher oxygen consumption rates for males than for females as well as higher dehydrogenase activity (Fuhrman and Fuhrman, 1959). Presumably, these higher metabolic activities reported for males indicate a higher degree of energy expenditure and hence a shorter life span.

Oviposition Rate

Temperature and relative humidity extremes have marked affects on reproduction and oviposition in arthropods (Bailey, 1969; Bateman, 1967; Burnett, 1956; Bursell, 1964a; Cloudsley-Thompson, 1970; Coombs and Woodroffe, 1962; Currie, 1967; Edney, 1954b; Goonewardene and Townshend, 1974; Gunn and Causeway, 1938; Hagstrum, 1973; Hagstrum and Leach, 1973; Hobson, 1938; Klomp and Gruys, 1965; Mueller and Stern, 1973; Smith, 1958; Surtees, 1964a; Wigglesworth, 1965). Some insect species exhibit a relatively wide temperature range over which they will lay eggs while others have a very restricted range. *Ptinus tectus* has been shown to oviposit at temperatures between 5°-30°C (Bursell, 1964a). The aphid, *Toxoptera graminum* will lay eggs over a temperature range of 5°-40°C (Mellanby, 1939). *Anopheles quadrimaculatus* will not oviposit at temperatures below 12°C (Cloudsley-Thompson, 1970). In general, it has been found that the oviposition rate in insects is maximal at temperatures which approximate the upper limit for reproduction; it decreases drastically at higher
temperatures and more slowly at lower temperatures (Bursell, 1964a; Chapman, 1971; Park and Frank, 1948).

The results shown in Figure 7, Table 10 and Table 11 for T. molitor indicate that a temperature of 10°C is sufficiently low to prevent oviposition regardless of the moisture conditions present. The egg-laying capacity of this species is greatest at 25°C/75% r.h., which again suggests that these values represent nearly optimal conditions for T. molitor. High temperature extremes (35°C) did reduce the mean number of eggs laid per female over a two day period, but not significantly (Table 11). With respect to moisture, dry-air conditions (12% r.h.) have a severely detrimental effect on the number of eggs deposited even at an optimal temperature of 25°C (Figure 7). This fits in well with the effects found for dry conditions on the survival capacity of the egg (Figure 3), larval, pupal and adult stages (Figure 4) of T. molitor as well as on adult longevity (Figure 6), and further substantiates the poor adaptability of this species to dry conditions.

Locomotor Activity

Changes in ambient temperatures effect ectothermic organisms both physiologically and behaviorally. Concomitant physiological changes in the animal occur because the ambient temperature can directly alter the metabolic rate of the organism at the cellular level (Anderson and Mutchmor, 1971;
Behavioral changes also take place as a result of both the physiological changes and the effects of temperature through specific sensory receptors on the central nervous system (Chapman, 1971; Colhoun, 1954; Fraenkel and Gunn, 1961; Prosser, 1955). The behavioral responses of ectotherms to changing temperatures frequently take the form of locomotor activities which result in the organism positioning itself in a region of more favorable environmental conditions (Andrewartha and Birch, 1954; Barlow and Kerr, 1969; Bentley, 1944; Bodenheimer, 1934; Clench, 1966; Cloudsley-Thompson, 1964; Crozier, 1924; Dainton, 1954; Deal, 1941; Fraenkel and Gunn, 1961; Fry, 1947; Heimburger, 1924; Kerkut and Taylor, 1958; Meyer and Raffensperger, 1974; Miller, 1929). Although there is a great deal of information concerning the effects of temperature on the physiology of insects, much less is known concerning its effects on behavior. In addition, there is not much known concerning what specialized temperature receptors, if any, insects possess. Several trichoid sensilla located on the antennae of Rhodnius and Melanoplus have been implicated as temperature receptors (Wigglesworth, 1965), and ablation experiments have suggested the location of temperature receptors on the antennae of other insects (Bursell, 1964a), but very little substantiating electrophysiological evidence for such receptors is available (Chapman, 1971). In some
insect species, the sensitivity to temperature appears to be distributed over the entire body surface although the tarsi and antennae seem to be more sensitive than other areas (Chapman, 1971; Cloudsley-Thompson, 1970). Electrophysiological studies on the tarsal nerve of P. americana indicate that neuronal discharge varies with temperature (Precht, 1968) thereby suggesting the presence of a specialized temperature receptor in the tarsus. Similar findings have been reported for gastropods and crustaceans (Kerkut and Taylor, 1958). It has also been shown that the labellar chemoreceptors of several Diptera appear to be sensitive to changes in temperature (Chapman, 1971; Fraenkel and Gunn, 1961) as their rate of neuronal discharge varies directly with temperature. It has also been suggested that unspecialized nerve endings in the integument may be involved with the peripheral reception of temperature (Bodenheimer, 1934; Bursell, 1964a; Chapman, 1971; Clarke, 1960; Crozier, 1924; Dainton, 1954).

With respect to moisture and the desiccating effects of a dry atmosphere, it is of paramount importance that insects be sensitive to existing moisture conditions and respond accordingly. Most insects have a range of preferred humidity at which they are relatively inactive (Bursell, 1964b; Flemister, 1964; Chapman, 1971; Fraenkel and Gunn, 1961). Deviations from this preferred humidity range in either direction generally result in increased locomotor activity (Barlow
and Perttunen, 1966; Cloudsley-Thompson, 1970; Fraenkel and Gunn, 1961). Specialized humidity receptors have been described for several insects and other arthropods. In *Tribolium castaneum* the branched peg organs of the basiconic sensillum type have been identified as humidity receptors (Fraenkel and Gunn, 1961; Roth and Willis, 1950). Similar hygroreceptors have been identified for other insects (Bursell, 1964b; Chapman, 1971; Roth and Willis, 1951a; 1951b) and arachnids (Fraenkel and Gunn, 1961; Lees, 1947).

In the present study, the effects of temperature and moisture on the burrowing activity of *T. molitor* larvae and the running activity of adults were investigated. The negative phototactic response of this species has already been established (Howard, 1955; Yinon, 1970), and the speed at which larvae would burrow and adults traverse a runway in order to avoid an adversive light stimulus was used as the index of locomotor activity.

The results shown in Figure 8 and Figure 9 indicate that relative humidity has little effect on the burrowing speed of *T. molitor* larvae or the running speed of the adults. It can also be seen that burrowing speed increased with increasing temperature from 10°-35°C and this agrees with the general effects of increasing temperatures on activity reported for ectotherms in general (Bullock, 1955; Bursell, 1964a; Cloudsley-Thompson, 1964, 1970; Fry, 1947; Kerkut and Taylor,
Locomotor activity for the larvae and adults was significantly reduced by exposure to a cold temperature extreme (10°C) for 1-6 hours as compared to that found under more optimal temperature conditions (25°C). However, after 12 hours of exposure to 10°C, the burrowing speed of the larvae and running speed of the adults at 25°C had increased significantly (Table 12 and Table 13), and after 24 hours their performance was very nearly identical to that at 25°C. This is a vivid demonstration of thermal compensation to a normally inhibitory cold temperature after only 24 hours and illustrates the temperature compensation potential of this species.

It is well known that the ability of ectotherms to adjust to changes in temperature is predicated upon their ability to maintain normal metabolic processes so that sufficient energy expenditure for locomotor activity and other vital processes is continued. Metabolic adaptations of ectotherms to changes in temperature are known to occur through shifts in the metabolic rate - temperature curve (translation) either to the right for high temperatures or to the left for low temperatures (Belehradek, 1957; Cloudsley-Thompson, 1970; Hochachka, 1971; Mutchmor and Richards, 1961; Rao, 1967) or by changes in the slope (rotation) of this curve (Clarke, 1967; Mutchmor,
1967; Richards, 1956). These compensatory responses of metabolic processes exhibited by ectotherms are reflected at the molecular level by the acclimation of enzyme activity (Anderson and Mutchmor, 1971; Fry and Hochachka, 1970; Somero and Hochachka, 1971). This acclimation of enzyme activity is attained by the induction of multiple molecular forms (isozymes) of the enzyme in question in such a way that the affinity of this enzyme for its substrate is greater at a particular temperature than would be the normal form of the enzyme. In other words, the basic process of temperature acclimation is the synthesis of new enzyme variants (isozymes) which are functionally better adapted and hence facilitate metabolic activities so that the organism may remain active at new temperature regimes (Aleksiuk, 1971; Baldwin and Hochachka, 1970; Fry and Hochachka, 1970; Harris, 1961; Haschemeyer, 1969; Heber et al., 1973; Katzen et al., 1970; Kimura, 1968; Koshland and Neet, 1968; Markert and Whitt, 1968; Newell and Northcroft, 1967; Somero et al., 1968; Wagner and Selander, 1974; Webb, 1964). Presumably, the induction of apyrase enzyme variants accounts for the enhanced physiological performance of _T. molitor_ larvae and adults after exposure to 10°C for 24 hours. The apyrase system is considered to be a major source of energy for muscular activity in insects and earlier investigations by Mutchmor and Richards (1961) established a correlation between the temperature coefficients
of apyrase activity and adaptation to different temperatures shown by T. molitor as well as by the housefly, M. domestica, the sarcophagid, Sarcophaga bullata, the waxmoth, Galleria mellonella, the orthopterans, Blaberus craniifer and P. americana, the crayfish, Cambarus diogenes, and the horseshoe crab, Limulus polyphemus. The results of the present study illustrate the capacity of T. molitor to adapt relatively quickly to shifts in temperature and as a result continue normal physiological performance at extremes of temperature. This does not imply that cold temperatures (10°C) do not constitute conditions of stress for this species. The data shown in Figure 4 for survival capacity in fact point out the limiting effects of temperature and moisture extremes. However, the ability to rapidly compensate for decreasing temperature and maintain normal locomotor activities has obvious survival value in that it provides the insect with the means by which it can relocate itself to regions of more favorable environmental conditions. In addition, this ability to compensate reflects important evolutionary ramifications. Some investigators have suggested that temperature compensation represents a form of phenotypic variation which may be an initial step in species formation (Anfinsen, 1959; Bateman, 1967; Behrisch, 1972; Bullini and Coluzzi, 1972; Cloudsley-Thompson, 1970; Haites et al., 1972; Kimura, 1968; Prosser, 1955). That is a population phenotypically adapted to inhibit
the limits of its range will be more conducive to genetic variations which coincide with its phenotypic ones. Phenotypic adaptation thereby increases the probability of fixation of a genetic variation since such a genetic change might not be adaptive in other populations of the species. Locomotory acclimation, as found in this experiment, is an example of phenotypic adaptation and may have considerable effects on the evolution of a species. The ability of a species to become behaviorally acclimated to changing environmental temperatures indicates that it possesses some of the phenotypic adaptability necessary for the evolution of a new species biotype possessing physiological mechanisms which enable it to cope with temperature extremes and eventually extend its ecological distribution.

Dispersion Behavior

The purpose of these experiments was to ascertain the effects of temperature and thermal acclimation on the dispersion (spatial distribution) of larval and adult *T. molitor* in a three-dimensional gradient. The results obtained for the dispersion of *T. molitor* larvae shown in Figure 10 through Figure 15 indicate a definite effect of temperature and thermal acclimation on their movement through grain. Larvae tested at 10°C without previous exposure to this temperature were found to aggregate in the middle layers of the cube.
(Figure 10) and away from the peripheral compartments (Figure 11). Aggregation has been reported to be a behavioral mechanism of thermoregulation utilized by insects under conditions of cold-temperature stress (Cloudsley-Thompson, 1964, 1970; Gaul, 1952; Gray, 1948; Hamilton, 1971; Kevin and Short-house, 1970; Lindauer, 1954a). Since the grain had been conditioned at 10°C for one week prior to testing and the temperature at the core of the grain bulk was nearly the same as the peripheral temperature (10°C), it is doubtful that there was any insulative effect in the central area of the cube. Most likely, cold temperatures stimulate individuals of this species to aggregate. However, larvae acclimated at 10°C for 24 hours prior to testing at the same temperature exhibited a dispersion pattern (Figure 10 and Figure 14) very similar to that shown by larvae under a more optimal temperature regime of 25°C (Figure 10 and Figure 12). This dispersion pattern was characterized by an initial downward movement of the larvae into the bottom vertical layer. A similar pattern was observed for larvae tested at 35°C, only in this case the initial downward dispersion was more rapid at this higher temperature. This correlates well with the stimulatory effect of high temperature on the locomotor activity of T. molitor larvae and adults reported in the previous section. Larvae acclimated at 35°C for 24 hours prior to testing (Figure 10 and Figure 15) exhibited a dispersion pattern which was some-
what more erratic although the tendency toward downward movement was still evident. This rapid dispersion pattern at 35°C may also reflect the drier moisture conditions present at this temperature. The results of Figure 16 show that the moisture content of the grain at this temperature is only 8% which constitutes a very dry condition. Thus, the dispersion pattern of the larvae may be a reflection of both the high temperature and low moisture extremes. At 25° and 10°C, the moisture content of the grain is substantially higher (14-15%) and therefore moisture does not constitute a condition of stress at these temperatures and test conditions. It should also be pointed out that the dispersion pattern exhibited by the cold-acclimated group (10°a/10°t) as contrasted with that shown by the warm-acclimated group (25°a/10°t) is in general agreement with the findings reported by Ernst and Mutchmor (1969) for the dispersal of stored-product insects in one plane. These authors showed that cold-acclimated larvae and adults of T. molitor, T. confusum and Trogoderma parabile demonstrated greater dispersal at low temperatures than did warm-acclimated insects. This tendency toward greater temperature independence shown by cold-acclimated animals has been verified for numerous ectotherms (Bullock, 1955; Colhoun, 1954, Cloudsley-Thompson, 1970; Precht, 1968; Prosser, 1955, 1961; Rao, 1967; Somero and Hochachka, 1971).
The tendency shown by *T. molitor* larvae toward downward movement suggests that this species exhibits a positive geotactic response with respect to its dispersion in grain. For most organisms the gravitational plane represents a basic plane of reference during locomotor activities. Some organisms move directly along the lines of gravitational force towards the center of the earth (positive geotaxis) whereas others orient away from it (negative geotaxis). It is well known that insects possess specialized gravity receptors. These gravity receptors are extremely diverse and differ from the statocyst gravity receptors of other organisms (Fraenkel and Gunn, 1961). Gravity receptors in many of the Hymenoptera consist of tufts of setae located between the head and thorax (Marler and Hamilton, 1966). The effective stimulus appears to be movements of the head in relation to the thorax. Similar receptors have been reported for several species of Odonata and Coleoptera (Fraenkel and Gunn, 1961). Although specific gravity receptors have not yet been identified for *T. molitor*, the disposition for downward movement (positive geotaxis) demonstrated for this species strongly implicates the presence of such receptors. The downward movement exhibited by the insects in this experiment is in direct contrast with the previous investigations of Hines (1958) who reported a negative geotactic response for *T. molitor* larvae under dry conditions. It is clear that further investigations are
necessary in order to ascertain the definitive response of this species to gravitational forces.

The results obtained for the dispersion of T. molitor adults shown in Figure 17 through Figure 22 also indicate a relationship between temperature and thermal acclimation on their movement through grain. Under normal conditions (25°a/25°t) the dispersion pattern was one of definite upward movement (negative geotaxis) into the top vertical layer (Figure 17). Adults tested at 10°C, however, without previous exposure to cold temperatures (25°a/10°t) were found largely in the middle layers of the cube and away from the periphery (Figure 18). This aggregation of adults at cold temperatures is another example of a behavioral thermoregulatory mechanism. However, after exposure to 10°C for 24 hours prior to testing, the dispersion pattern showed an acclimatory response in that the adults were again found to move into the remain in the upper vertical layer of the cube (Figure 17 and Figure 21) as was found for the adults tested at 25°C. Thus, after only 24 hours, complete temperature acclimation to 10°C had occurred and the adults were moving as they would at 25°C. The dispersion pattern of these insects at 35°C without previous exposure to this high temperature was very similar to that exhibited by insects under more normal and optimal conditions (25°C). In fact, there was no significant difference in the vertical layer/temperature interaction between the 25°a/25°t
and 25°a/35°t, 25°a/25°t and 35°a/35°t, 25°a/35°t and 35°a/
35°t, and 10°a/10°t and 35°a/35°t groups (Table 19). In all
cases, very few adults were found in the middle-bottom and
bottom vertical layers (Figure 17, Table 16) regardless of
temperature. This again points to a negative geotaxis for the
adults of this species and is in general agreement with the
investigations of Hines (1958) who reported a negative geo-
tactic response for T. molitor adults. Thus, the adults
exhibit a negative geotaxis in contrast to the positive geo-
taxis found for the larvae in this experiment. This suggests
that the geotactic response of this species undergoes a
reversal during metamorphosis which is presumably caused by
the extensive neural reorganization taking place at this time
(Wigglesworth, 1965).

Previous investigations on the movement of stored-product
insects through bulks of grain indicate that there is a great
deal of variation in dispersion patterns between different
species. Larvae and adults of the grain weevils, Calandra
granaria and C. oryzae, were found to exhibit downward move-
ment in an experimental grain tower (Howe, 1951). However,
this tendency was much more pronounced in C. granaria which
is smaller in size than C. oryzae. Howe (1951) suggested that
size may be an important factor in the movement of insects
through grain. Investigations on Tribolium castaneum adults
(Surtees, 1964c) indicate that these tenebrionids are also
surface dwellers as was the case with *T. molitor* in the present study. Similar results for *T. castaneum* have been reported by Amos and Waterhouse (1969) and Hagstrum (1973), as well as for adults of *Oryzaephilus surinamensis* (Amos, 1968; Surtees, 1964b), *Sitophilus granarius* (Smerka and Hodson, 1959; Surtees, 1964b) and *Lasioderma serricorne* (Lefkovitch and Currie, 1967). On the other hand, adults of *Lepinotus patruelis* (Fahy, 1971) and *Rhyzopertha dominica* (Surtees, 1964b) exhibited a strong tendency to move into the remain in the center of the grain bulk under all test conditions of temperature and moisture. Larvae of *Ptinus tectus* (Bentley, 1944) and *S. granarius* (Bailey, 1969; Surtees, 1963, 1964b) showed a definite deviation from homogeneous dispersion in that they were found to concentrate at the periphery of the grain bulk at all vertical levels. In the present study, neither the larvae nor the adults of *T. molitor* showed a tendency to concentrate in the corner units or central units of the cube; that is, horizontal dispersion was relatively homogeneous in all cases. However, vertical dispersion was not homogeneous in that the adults were found to largely occupy the top vertical layer, and the larvae the middle-bottom and bottom vertical layers. Larvae and adults of *Sitophilus oryza* (Robinson, 1926) and *Cryptolestes ferrugineus* (Surtees, 1964b) exhibit homogenous dispersion both vertically and horizontally. Variations in dispersion patterns found
between different species are not surprising if it is kept in mind that patterns of dispersion are spatial expressions of responses to environmental stimuli within the grain and various species will respond differently to gradients of temperature and moisture, gravitational forces and in the nature of their acclimatory responses.

The results of these dispersion studies (Figure 10 and Figure 17) offer some suggestive control implications in conjunction with the survival capacity data (Figure 4). The viability of this species was found to be severely reduced under dry environmental conditions (12% r.h.) and high (35°C) and low (10°C) temperature extremes. Thus, the heating and drying of commercial grain before storage would be an effective method of reducing the survival capacity of this species and other stored-product insects thereby minimizing their density in stored grain bulks. However, the economic feasibility of the refrigeration or heating and drying of grain silos as contrasted to the actual monetary damage caused by the insects remains to be analyzed.

General Discussion and Conclusions

The object of the present study was to accurately define the temperature relationships of *T. molitor* within the framework of stringent relative humidity controls which were frequently lacking in the earlier investigations on the effects of temperature on terrestrial arthropods. The
biological parameters studied, which include survival capacity, egg-laying capacity, adult longevity, changes in larval temperature tolerance, locomotor activity and dispersion behavior, were chosen for two reasons. First, they represent biological and behavioral parameters which are of paramount importance to the success of the species and in understanding the ecology and distribution of this insect. Second, they represent areas which have not been thoroughly investigated for this species in previous studies.

The results obtained in this study support the following conclusions:

(1) Embryological development is successful at 25°C/75% r.h. and 35°C/75% r.h., with development more rapid at 35°C (15 days) than at 25°C (19 days). Under these environmental conditions, the eggs show an initial period of more rapid water uptake (1-4 days) followed by a period of reduced water uptake (5-10 days).

(2) Dry-air conditions (12% r.h.) result in a progressive loss of water from the egg and eventual mortality due to desiccation.

(3) At a cold temperature of 10°C water absorption is minimal and embryological development is not completed.

(4) A temperature of 25°C does not constitute a condition of stress for the larvae, pupae and adults of this species even at extremes of relative humidity and long periods of
exposure.

(5) Extremes of temperature (10° and 35°C) do not result in significant mortality under relative humidity conditions of 52% and 75%. Thus, 25°C represents a nonstressful temperature at all conditions of humidity, and 52% and 75% r.h. represent nonstressful moisture levels within a temperature range of 10°-35°C.

(6) The deleterious effects of temperature and relative humidity extremes are magnified at long periods of exposure (24-48 hours).

(7) Under dry conditions (12% r.h.), cold-temperature extremes result in a slightly higher mortality rate for young larvae than do high-temperature extremes (35°C). The opposite effect was found for the older larvae.

(8) The pupal stage of *T. molitor* is the most resistant to temperature and relative humidity extremes whereas the egg and young larval stages are the least resistant.

(9) The mortality of older larvae at extremes of temperature is higher under moist conditions (98% r.h.) than it is under dry conditions (12%), especially at high stressful temperatures (35°C).

(10) High temperature extremes (35°C) reduce the survival capacity of this species more so than do low temperature extremes at all humidities and periods of exposure.
(11) There is a progressive increase in resistance to high lethal temperatures (42°C) throughout larval development. This increase in survival time is manifested under dry and moist conditions.

(12) Under extremely dry conditions (12% r.h.), the greatest resistance to a lethal temperature of 42°C is shown by older and larger larvae (instars 16-27); the least resistance is shown by smaller larvae (instars 1-15).

(13) Adult females of *T. molitor* exhibit longer life spans than do males of this species at all temperatures.

(14) The longevity of adult males and females is shortest at high temperatures (35°C) regardless of humidity.

(15) Dry conditions have the most deleterious effect on adult longevity.

(16) The highest oviposition rates were found at temperatures of 25°C and 35°C, and at relative humidities of 75% and 98%. Low relative humidity (12%) severely reduced the number of eggs laid per female over a 48 hour period at these temperatures.

(17) No eggs were deposited by *T. molitor* females at 10°C.

(18) Exposure to a temperature of 10°C for 1-6 hours reduced the burrowing speed of warm-acclimated (25°C) larvae and the running speed of warm-acclimated adults. However, acclimation at 10°C for 24 hours resulted in levels of performance very nearly identical to those found under a more
optimal temperature of 25°C.

(19) The most rapid burrowing and running speeds were found at the higher preconditioning temperature (35°C).

(20) The results suggest that a temperature of 25°C and a relative humidity of 75% represent very nearly optimal conditions for this species.

(21) The dispersion pattern of the larvae at 25° and 35°C is characterized by initial downward movement (positive geotaxis) into the bottom vertical layer of the dispersion cube. At 10°C, there is a strong tendency to remain in the middle vertical layers. After acclimation to 10°C for 24 hours the dispersion pattern is very similar to that shown at 25°C.

(22) The adult dispersion pattern at 25° and 35°C is characterized by a strong tendency of the adults to move into and remain in the top vertical layer of the cube (negative geotaxis). At 10°C, the adults remain in the middle layers. However, after a 24-hour acclimation period at 10°C, the normal dispersion pattern returns.

(23) The larvae and adults exhibit a homogeneous horizontal dispersion within the cube, and a heterogeneous vertical dispersion in that the adults are surface dwellers and the larvae bottom dwellers.
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