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INVESTIGATIONS INTO THE COLD RESISTANCE OF THE EGGS AND LARVAE OF *BOOPHILUS DECOLORATUS* (KOCH, 1844), *BOOPHILUS MICROPLUS* (CANESTRINI, 1888) AND *MARGAROPUS WINTHEMI* KARSCH, 1879

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INTRODUCTION

The continued existence of an organism is dependent upon its ability to tolerate a set of environmental conditions whose component factors may vary qualitatively or be present in excess or be deficient. Any influence falling outside the tolerance range of an organism becomes a limiting factor for its survival (Shelford, 1913; Odum, 1963).

Of all these limiting factors climate plays the most important role and determines the ecological and geographical distribution and the seasonal activities of a species (Imms, 1931; MacLeod, 1936). Within the climate complex temperature plays a fundamental role, a species being confined within a specific temperature range (Odum, 1963). An environment favourable to a given species can be determined by the temperatures prevailing in the biotopes specific to the organism. Not only the average daily and the average yearly temperature, but also its range of variations are reflected in the physiology of the given species (Andrewartha & Birch, 1954). Theiler (1948) lists the following as limiting factors for ticks:—

(*a*) the soil, its texture, pH value, humidity and temperature;

(*b*) sunlight, its intensity and duration;

(*c*) air temperature and the intensity or length of the periods of frost;

(*d*) rainfall, its amount and spread taken in conjunction with the length of the intervening dry periods;

(*e*) the vegetation types which can be taken as a summation of the climatological and physiographical factors of a locality.

To date the resistance and reactions to temperatures below freezing point have been studied in a few ticks only. A summary of the published literature is given in Table 1.

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TABLE 1.—*Cold tolerance of ticks as reported in the literature*

Tick Species	Exposed to temperature range	Egg	Larva	Nymph	Adult	Develop-ment	References
	0° C to −5° C						
Boophilus annulatus.					−(*e*)		Cotton, 1915
Boophilus spp.			−(u)		−(u)		Enigk, 1954
Dermacentor marginatus.					+		Yashkul, 1960
	−5° C to −10° C						
Ixodes ricinus.			+(u)	+(u)	+(u)		MacLeod, 1935
Dermacentor variabilis.					−(e)		Mail, 1942*
Ixodes californicus.					−(e)		Mail, 1942
Ixodes texanus.					−(e)		Mail, 1942
Hyalomma savignyi.			+(u)		+(u)		Enigk, 1954
Boophilus microplus.		+	+		+(e)	+	Hitchcock, 1955
Ixodes kingi.			+(u)	+(u)			Elzinga & Rees, 1960
Ixodes kingi.			+(e)	+(e)			Elzinga & Rees, 1960
	−10° C to −15° C						
Ixodes ricinus.					+		MacLeod, 1936
Dermacentor variabilis.					−(u)		Mail, 1942
Haemaphysalis cinnabarina.					+(e)		Mail, 1942
Ixodes californicus.					−(u)		Mail, 1942
Hyalomma dromedarii.			−	−	−		Enigk, 1944
Boophilus calcaratus.			−	−	−		Enigk, 1944
Rhipicephalus sanguineus.					+(u)		Enigk, 1944
Rhipicephalus bursa.			+(u)	+(e)	+(u)	+	Enigk, 1944
Rhipicephalus bursa.					−(e)		Enigk, 1944
Dermacentor pictus.					+(e)		Enigk, 1944
Rhipicephalus bursa.			+(u)		+(u)		Enigk, 1954
Dermacentor pictus.			+(u)		+(u)		Enigk, 1954
	−15° C to −20° C						
Boophilus annulatus.			−				Cotton, 1915
Dermacentor variabilis.					+		Knipling & Sullivan, 1957
	−20° C to −30° C						
Dermacentor variabilis.			+	+			Bishopp & Smith, 1938
Dermacentor variabilis.		−					Mail, 1942
Ixodes texanus.					−(u)		Mail, 1942
Dermacentor variabilis.			+(u)	+(u)	+(u)		Smith *et al.*, 1946

Explanation of symbols:

"—" death; "(e)" engorged; "(u)" unengorged; "+" survival.

* Salt & Mail (1943) criticize the results of Mail (1942) pointing out that he considered the rebound point as the freezing point. Basing their argument on the findings of Salt (1936) they show that there can be a discrepancy of up to 25° C between these two values and that therefore the "freezing points" of Mail (1942) cannot be regarded as valid.

The present article is a report on an investigation into the temperatures below freezing point at which the eggs and the larvae of *B. decoloratus*, *B. microplus* and *M. winthemi* can survive, and their ability to develop further upon being returned to optimal conditions.

Materials and Methods

Eggs

Ten days after oviposition eggs (kept at 25° C and 90 per cent RH) from different females of the same species were pooled before being distributed into tubes, each of which received a representative batch of about 300 eggs. Groups of ten tubes per species were exposed for varying periods (the periods increasing in length by 24 hours) to temperatures of 0° C, —5° C, —10° C, —13° C to —15° C and —23° C. Tubes in Group I were exposed for 24 hours, in Group II for 48 hours, in Group III 72 hours *et sequitor* up to Group X exposed for 240 hours.

The control group was kept in the climate room (25° C and 90 per cent RH) throughout. After exposure to their respective experimental conditions the tubes were returned to the climate room, each tube was inspected daily until its first larval hatch was observed when it was set aside and left undisturbed.

In so far as the eggs of the three species are known to hatch in under 50 days to 60 days in the climate room the percentage hatch was checked when this period had lapsed after oviposition. The counts were made under the stereomicroscope, on filter paper marked out in grids and placed in a petri dish standing in a warm water bath.

Larvae

To obtain equal batches of larvae, the material was divided out at the egg stage, and the tubes left in the climate room for the eggs to hatch. The larvae were not used until they had hardened, had become active, had climbed up the side of their containers and had come to rest at the top in the plug of cotton wool. *M. winthemi* larvae, however, remained in a cluster at the bottom of their tubes, and showed no marked activity or inclination to travelling upwards. They were hence assumed to have " hardened " in the same period after hatching as had *B. decoloratus* and *B. microplus*. After exposure, as per experimental design, the larvae were returned to the climatic chamber; after 48 hours the live larvae in the various batches were counted, the same technique being used as for the egg counts. Larvae, which showed no signs of movement and in which the legs were held tightly against the body were considered to be dead.

Experimental Results and Discussion

The results clearly illustrate the effect of low temperatures upon the development and the length of developmental periods of the egg and the larva of *B. decoloratus*, *B. microplus* and *M. winthemi*; the three species are seen to show significant differences in their resistance and tolerance.

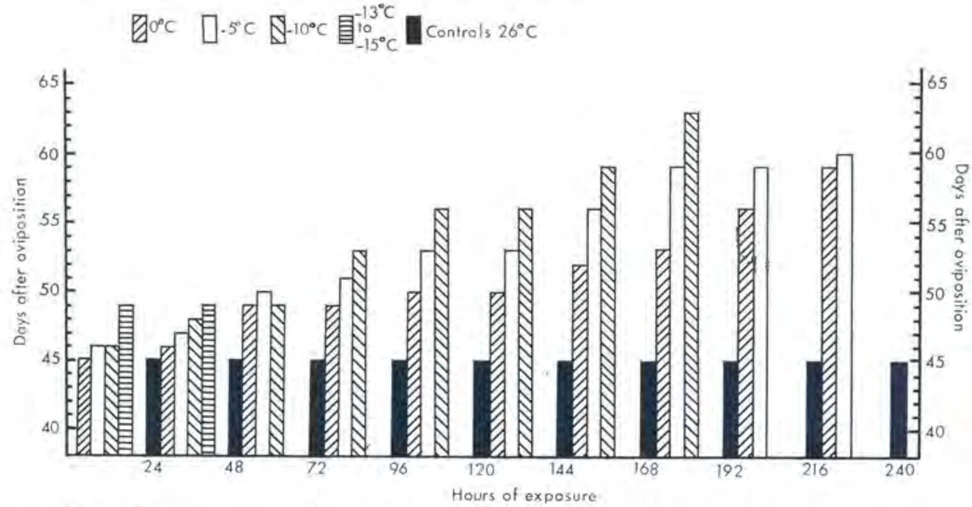

FIG. 1.—Time of larval hatchings of *Margaropus winthemi* after exposure of eggs to low temperatures

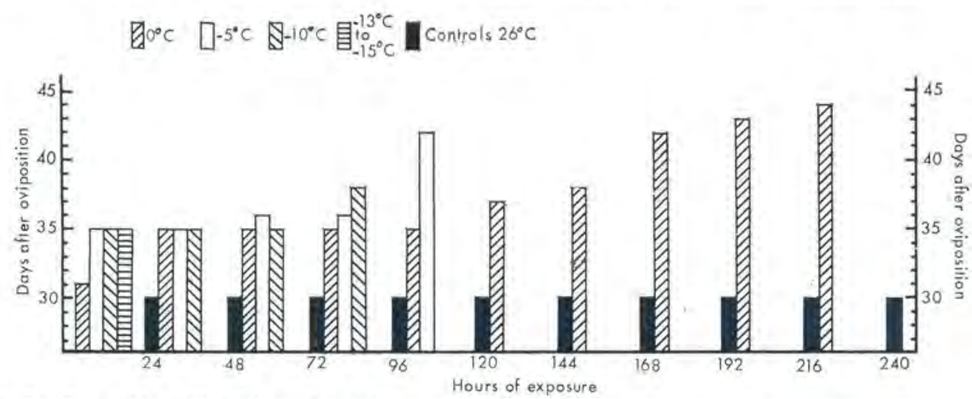

FIG. 2.—Time of larval hatchings of *Boophilus decoloratus* after exposure of eggs to low temperatures

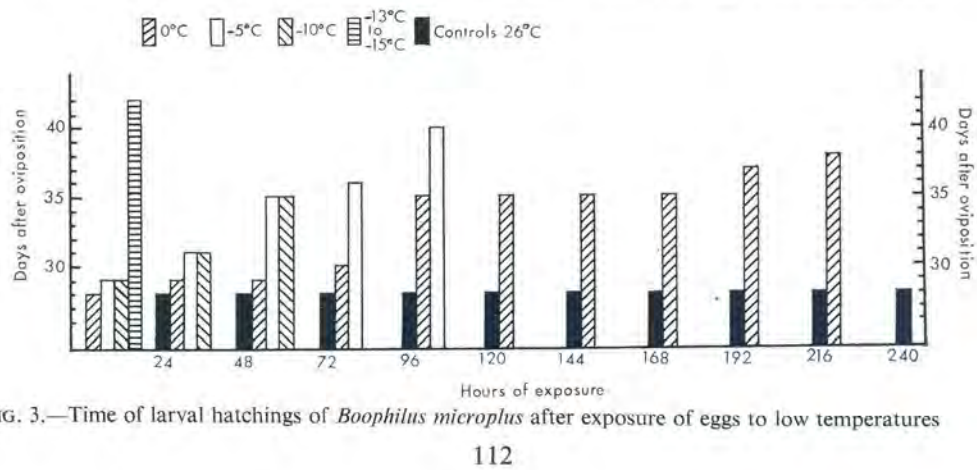

FIG. 3.—Time of larval hatchings of *Boophilus microplus* after exposure of eggs to low temperatures

Eggs (Fig. 1, 2, 3)

In the ten egg groups of *B. decoloratus*, *B. microplus* and *M. winthemi*, exposed ten days after oviposition to temperatures of 0° C, —5° C, —10° C or —13° C to —15° C, the time of exposure being increased by 24 hourly intervals, the first egg hatchings show a correlation with the temperature gradient and the length of exposure. In general it was found, that the hatchings were delayed progressively both with falling temperatures and with longer exposure until no further hatchings took place.

M. winthemi eggs tolerated the various low temperatures, especially in the range of —5° C, —10° C and —13° C to —15° C, considerably better than did those of the other two boophilids. *B. decoloratus* eggs were slightly more resistant than those of *B. microplus*. Eggs exposed to a temperature of —23° C did not hatch.

A comparison of the percentage hatch of the three species (Fig. 4, 5, 6) shows a marked difference in the degree of cold resistance between *M. winthemi* and the other two boophilids. When exposed to 0° C the percentage hatch is approximately the same up to the sixth day for all three species; after the seventh day the percentage hatch decreases considerably in *B. decoloratus* and *B. microplus* and only drops rapidly after the ninth day in *M. winthemi*. In the temperature range of —5° C, —10° C and —13° C to —15° C the difference is marked as from the first day; in *M. winthemi* the percentage hatch is considerably higher and hatching lasts for a much longer period than in the other two boophilids, the two latter differing but slightly.

Larvae (Fig. 7, 8, 9)

Upon exposure to the various low temperatures, *M. winthemi* larvae proved to be the most resistant, they tolerated temperatures of —10° C up to 144 hours and even —13° C to —15° C up to 48 hours. Larvae of *B. decoloratus* occupied an intermediate position, some larvae survived —10° C for 24 hours. *B. microplus* larvae were shown to be the most susceptible to low temperatures, they could tolerate a temperature of 0° C up to 72 hours only. The difference in tolerance between *B. decoloratus* and *B. microplus* larvae is significant. No larvae tolerated a temperature of —23° C.

Cold Tolerance and the Geographical Distribution of the three Species

The temperature gradient lines plotted on maps are at best approximate and generalized. Thus in the maps depicting the annual average frequency of days with minimum temperatures below 0° C and the duration of frost period, it must be borne in mind that within the temperature gradient lines there may be topographical variations; northern slopes may be warmer than southern slopes, that plant coverage slows down the loss of heat, and that air movement accelerates it. Hence within the frost zones there may be niches, which, though not presenting an optimal biotope, may yet fulfil the minimal ecological requirements for the development and maintenance of a given species.

Margaropus winthemi (Maps 1 and 2)

The experimental findings show that this tick, active during winter, is the most tolerant to cold. This tolerance is reflected in its distribution. It can maintain itself in areas of 90 days of frost spread over 180 days per year, and is present in the coldest zones of South Africa. It can thus be assumed that in these zones the tick is never exposed to temperatures lower than those tested in the experiment, nor is it exposed for such long periods. Cold is thus not a factor restricting its spread; other factors must play the limiting role, such as high humidity and high temperature, as suggested by Theiler & Salisbury (1958).

Boophilus decoloratus (Maps 3 and 4)

B. decoloratus though less resistant than *M. winthemi*, yet manages to maintain itself in areas within the zone of 90 days frost, spread over a period of 150 days per annum. Its experimentally proved slighter tolerance taken in conjunction with its distribution, suggests that within the larger macroclimates there are local topographical variations which may ameliorate conditions sufficiently to allow for its survival, though possibly in reduced numbers. (That slight local variations play a role in the time of egg-laying and survival of the engorged female has been shown by Kraft, 1961). The cold factor in the winter conditions obtaining in South Africa thus cannot be regarded as a factor restricting its spread, though cold may limit its numbers and its activity. It would seem, as suggested by Theiler (1949), that decreasing humidity plays the more important role in limiting its distribution.

B. microplus (Maps 5 and 6)

The experimental findings show that this exogenous tick is the least tolerant of the three species, and that the larvae are exceptionally susceptible to cold. They can only tolerate 0° C for 72 hours, and die when exposed to lower temperatures. The absence of *B. microplus* from a region may be ascribed either to its non-introduction or to its inability to establish itself after having been introduced. Although it is more prevalent in the milder coastal regions, it is also present in areas having up to 60 days of frost, spread over a period of up to 150 days per annum. Its presence within the cold highveld is in conflict with its known preference for warm, humid conditions in other parts of Africa, and also in conflict with the above experimental findings; as yet it is unknown how tolerant the adults are to cold. Once again localized microclimatic conditions, which offer minimal conditions for its survival, have to be postulated. In general, however, one can assume that cold does influence its spread and survival.

Summary

The experimental results show clearly, that low temperatures have a delaying effect upon the development of the free-living stages of *B. decoloratus*, *B. microplus* and *M. winthemi*.

The tolerance of eggs to low temperatures is greatest in *M. winthemi*, less and approximately equal in *B. decoloratus* and *B. microplus*.

The percentage of larval hatchings is greatest in *M. winthemi*, considerably less and approximately equal in the two boophilids.

Larvae of *M. winthemi* are most tolerant of low temperatures, *B. decoloratus* occupies an intermediate position, *B. microplus* being the most susceptible.

The experimental findings in conjunction with the geographical distribution indicate that cold is not a limiting factor in the spread of *M. winthemi*, that it exerts a slight influence on *B. decoloratus* by limiting its numbers, and that it undoubtedly plays a role in restricting the spread and survival of *B. microplus*.

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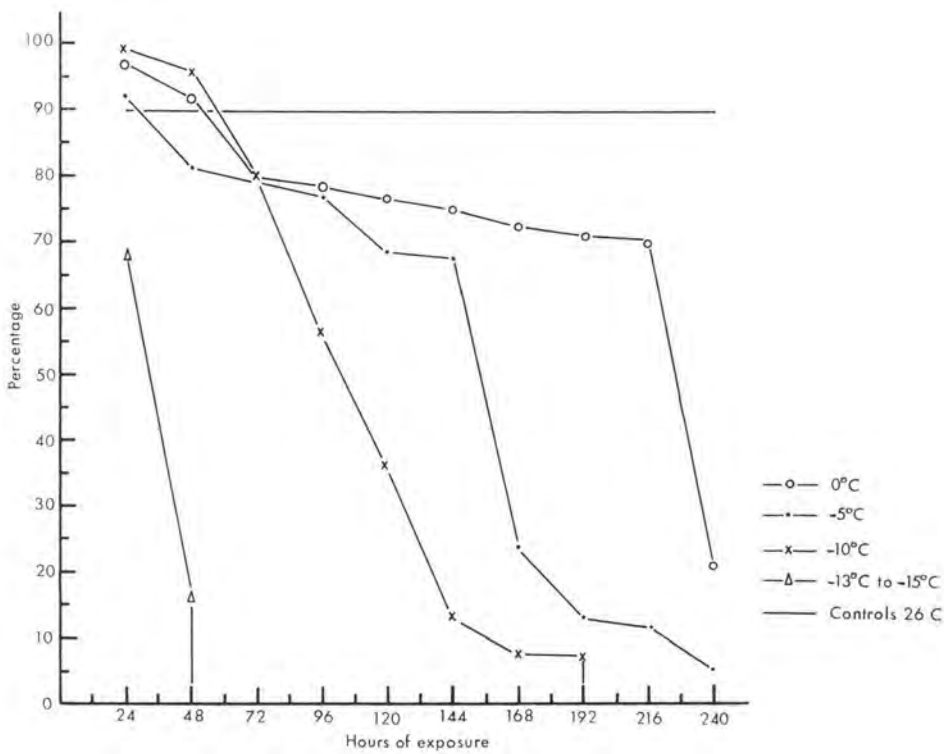


FIG. 4.—Percentage hatch of *Margaropus winthemi* eggs. exposed to low temperatures

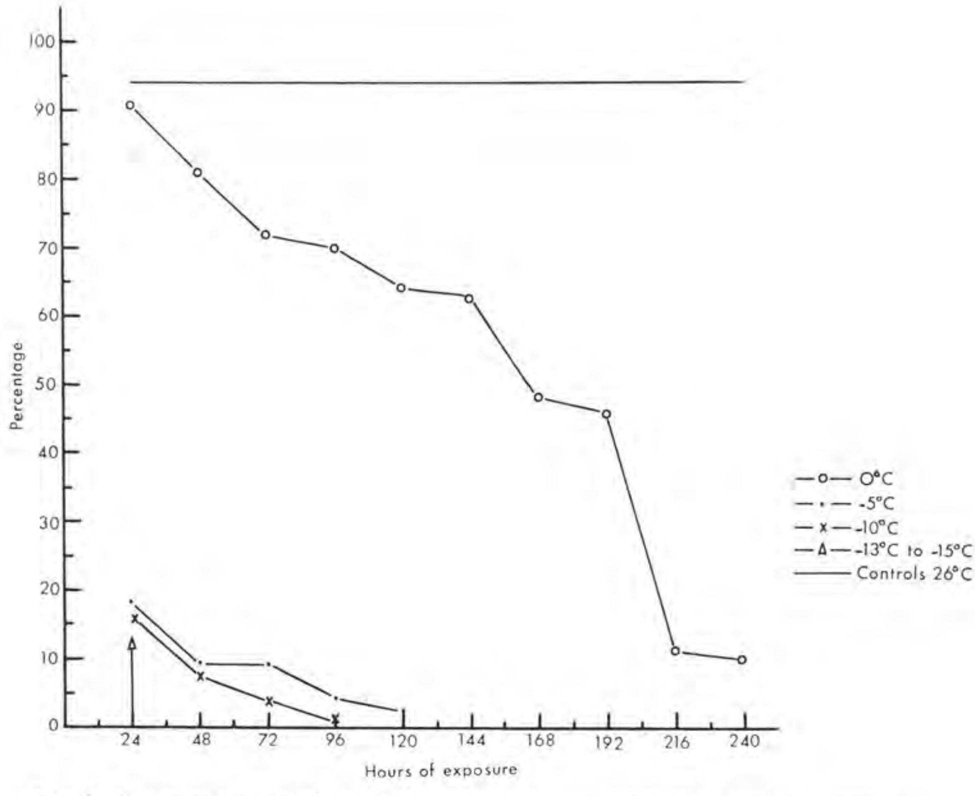


FIG. 5.—Percentage hatch of *Boophilus decoloratus* eggs, exposed to low temperatures

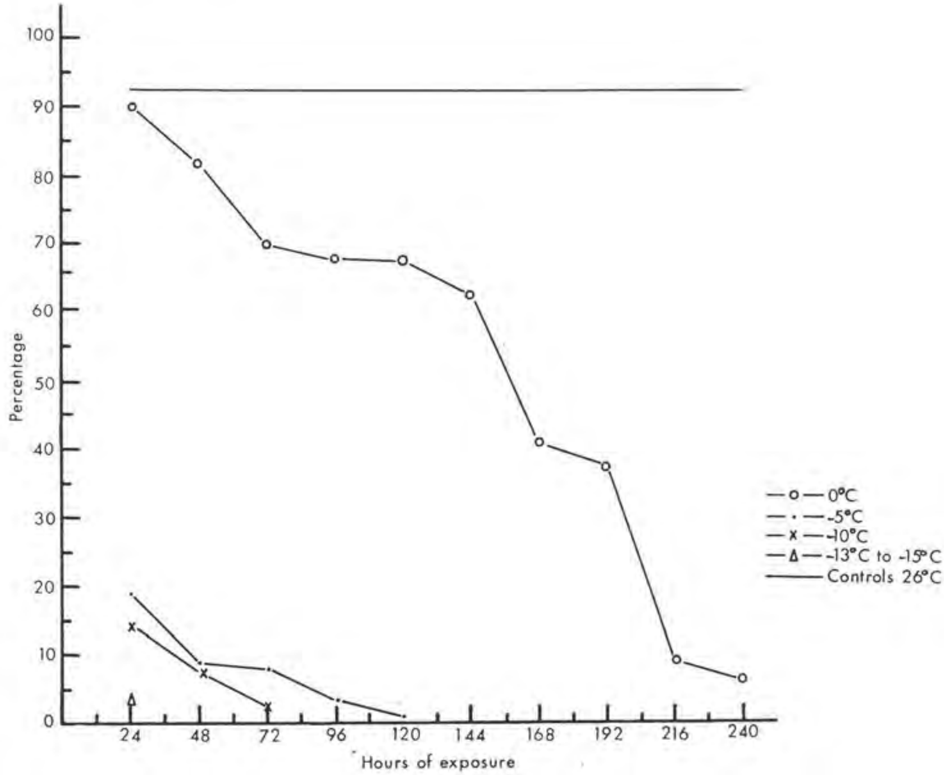


FIG. 6.—Percentage hatch of *Boophilus microplus* eggs, exposed to low temperatures

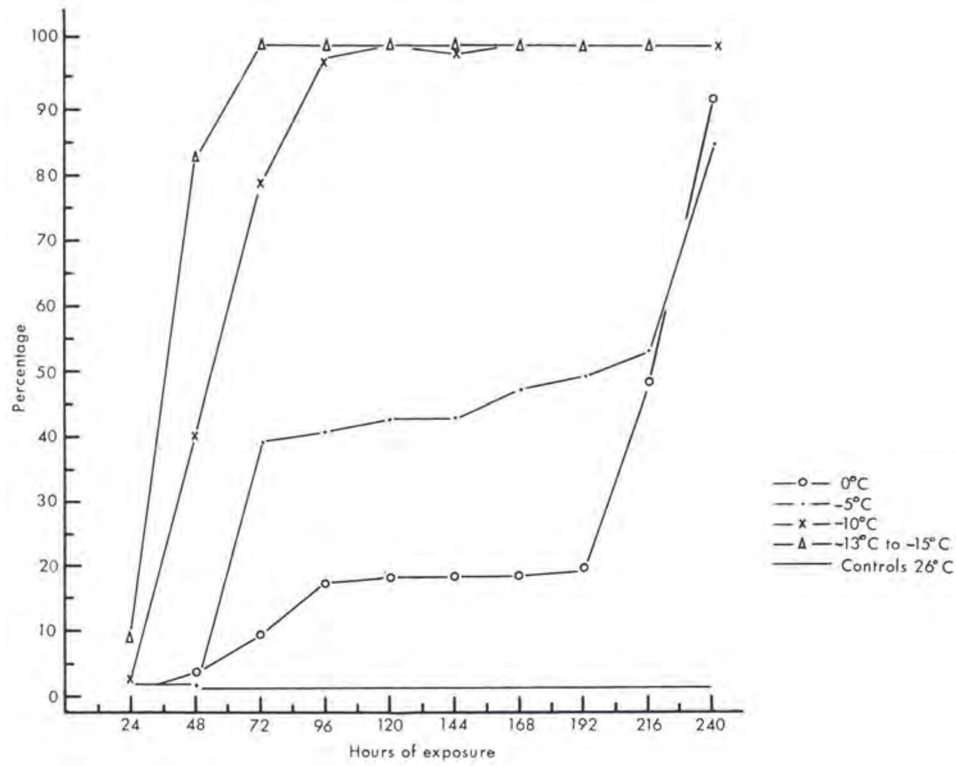


FIG. 7.—Death rate of *Margaropus winthemi* larvae, exposed to low temperatures

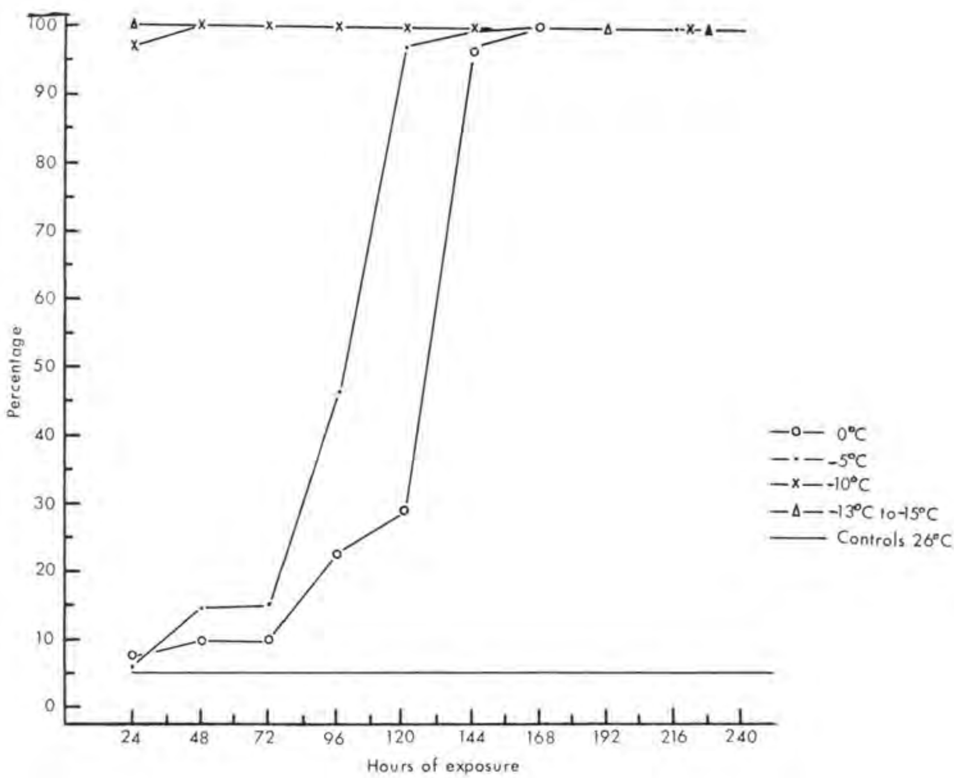


FIG. 8.—Death rate of *Boophilus decoloratus* larvae, exposed to low temperatures

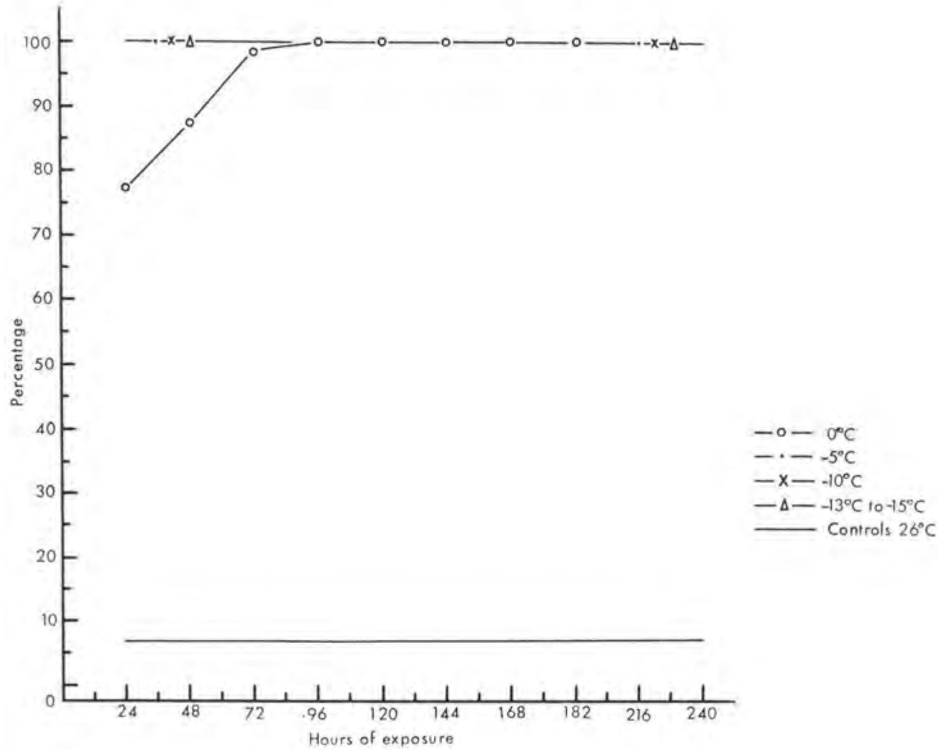
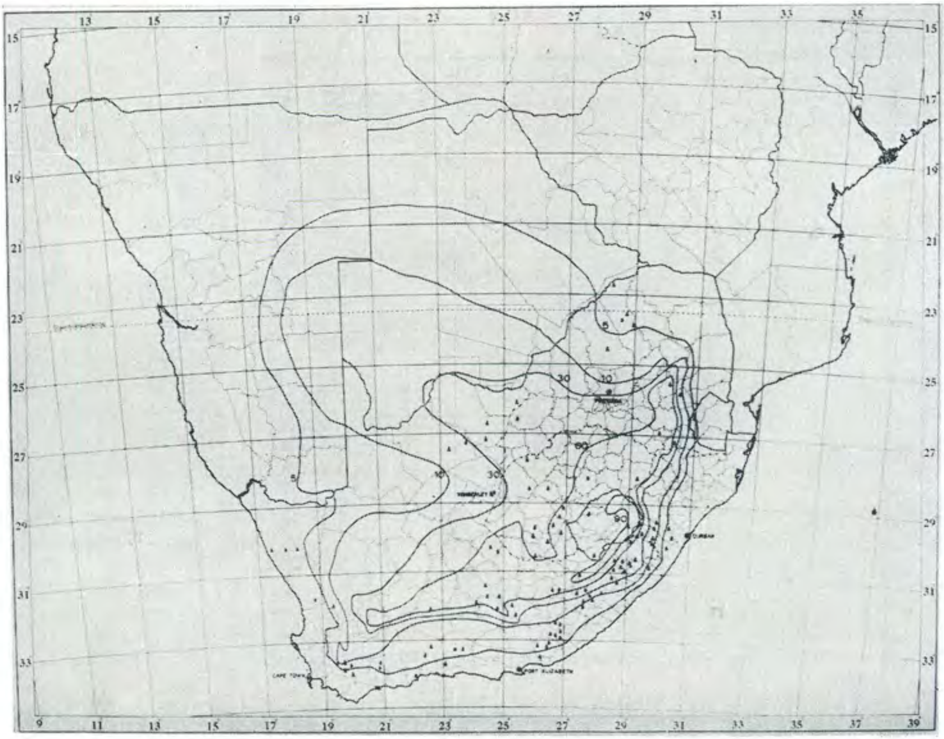
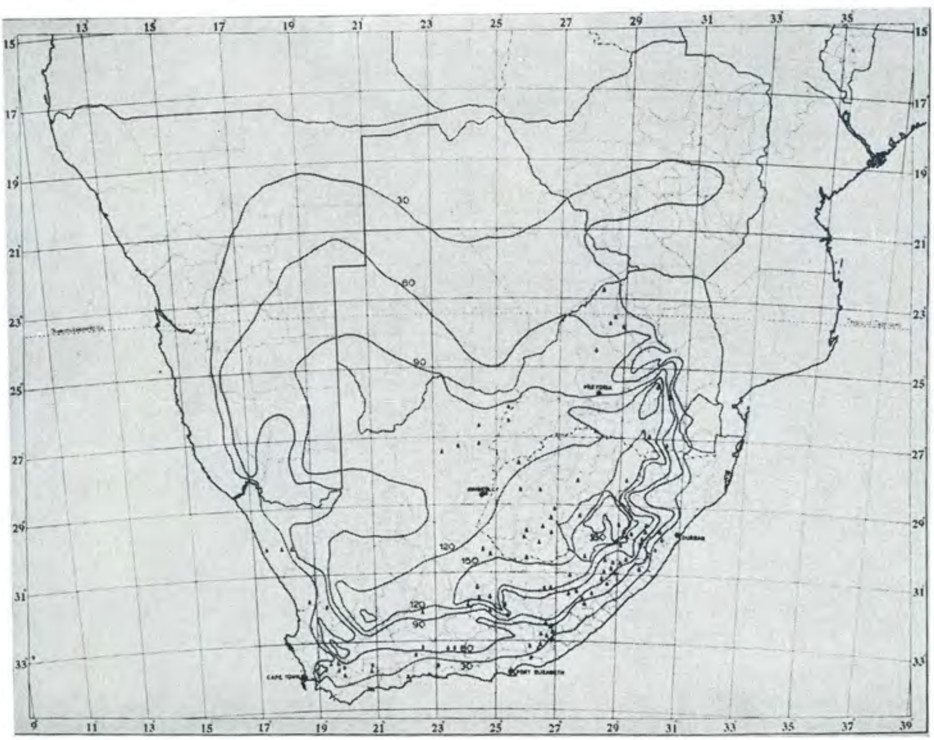


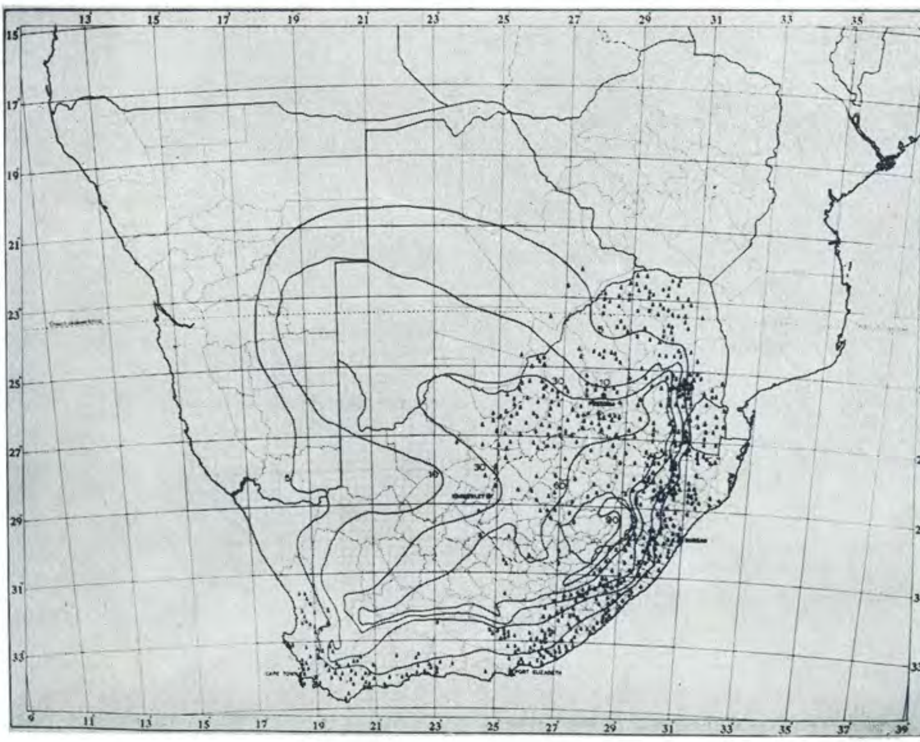
FIG. 9.—Death rate of *Boophilus microplus* larvae, exposed to low temperatures



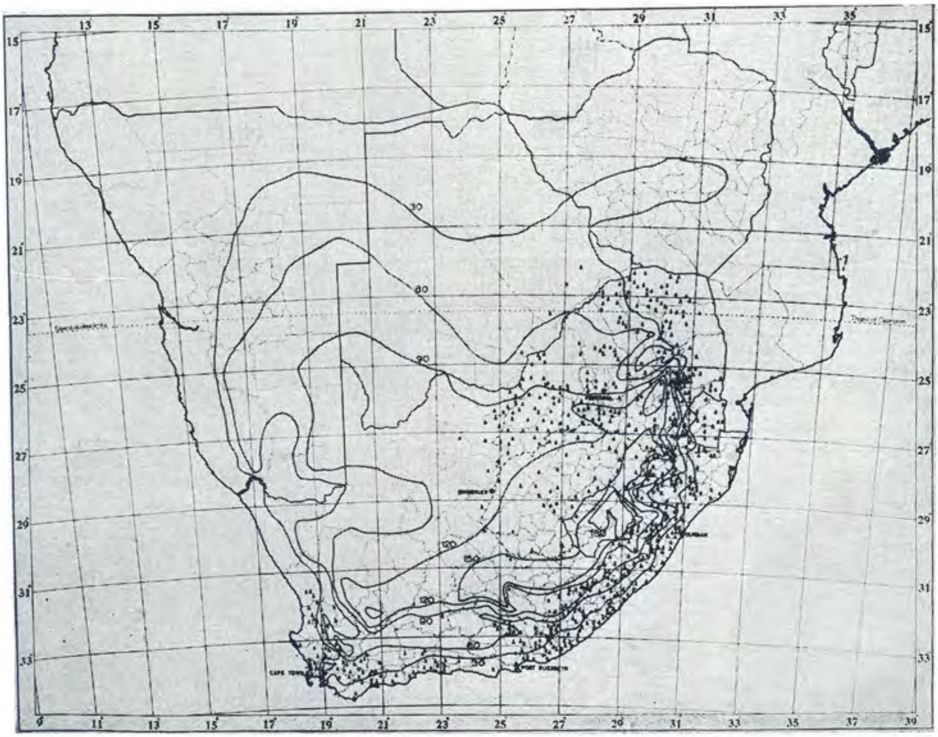
*MAP 1.—Distribution of *Margaropus winthemi* correlated with the average annual frequency of days with minimum temperature below O° C
* All maps based on Schulze (1965)



MAP 2.—Distribution of *Margaropus winthemi* correlated with the duration of the frost period (in days)

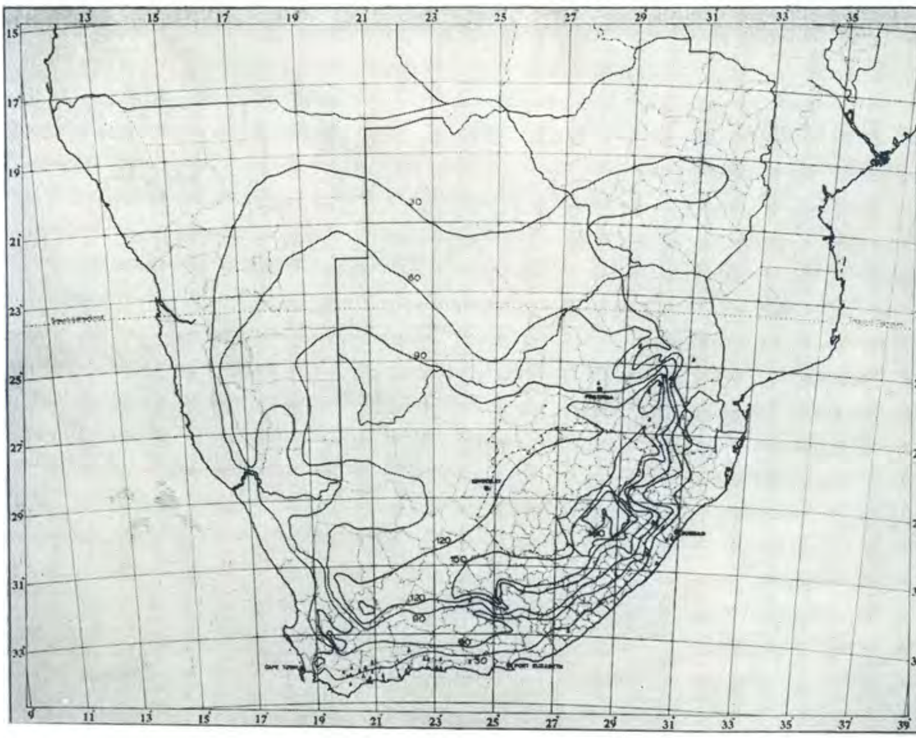


MAP 3.—Distribution of *Boophilus decoloratus* correlated with the average annual frequency of days with minimum temperature below O° C



MAP 4.—Distribution of *Boophilus decoloratus* correlated with the duration of the frost period (in days)

MAP 5.—Distribution of *Boophilus microplus* correlated with the average annual frequency of days with minimum temperature below 0° C



MAP 6.—Distribution of *Boophilus microplus* correlated with the duration of the frost period (in days)