Cold Hardiness in Overwintering Juvenile Grasshoppers[1]

GORDON ALEXANDER, Department of Biology,
University of Colorado

Most species of grasshoppers in cold or temperate regions pass the winter in the egg stage, but a few species overwinter as intermediate juvenile instars. While the embryonic stages are known to be tolerant of very low temperatures (Uvarov, 1966) apparently no studies of cold-hardiness in overwintering nymphs have been reported. The present paper summarizes observations on the extent and nature of such cold-hardiness.

Species with overwintering nymphs hatch in late summer, molt two or three times before the first freezing temperatures, and are usually in the third or fourth instar before the first severe cold spell. They complete their molts during the following spring, when increasing photoperiod seems as much a factor in stimulating molting as is increasing temperature (Halliburton and Alexander, 1964).

In the region around Boulder, Colorado, at least five species of Acrididae have this type of life cycle. One of these, *Psoloessa delicatula* (Scudder), is quite rare. Another, *Chortophaga viridifasciata* (DeGeer), is restricted to grassy areas below 7,000 feet in altitude and is only locally common. The other three, *Eritettix simplex* (Scudder), *Arphia conspersa* Scudder, and *Xanthippus corallipes* (Haldeman) are all relatively abundant and rather widely distributed. These three, hereafter referred to only by generic names, occur on the plains and up to various altitudes in the Front Range of the Rocky Mountains—*Eritettix* to 9,000 feet, *Arphia* to above 10,000 feet, and *Xanthippus* to timber line and above (11,000 feet). This report deals primarily with cold-hardiness in these three, last named species, with only an incidental observation on the other two.

One might assume that these overwintering nymphs are dor-

[1] This study was made possible by N.S.F. Grant G-5507 and a grant in aid from the Council on Research and Creative Work, University of Colorado, both here gratefully acknowledged.

mant ("hibernating") during the entire winter, but this is not the case. The young grasshoppers are periodically active, during mild weather, even when such weather follows cold periods of − 18° C (0° Fahr.) or lower. *Active* nymphs of the three commonest species have been collected near Boulder in every winter month. While undoubtedly protected by snow cover at times, they are exposed to extreme cold at other times—during periods of dry cold weather in which ground vegetation and soil surface are at temperatures far below 0° C—and these nymphs are most abundant on south facing slopes, where snow does not persist.

It is obvious that these juvenile grasshoppers are much more tolerant of low temperatures than are adult grasshoppers of typical summer species. A few individual adults survive until after the first "hard" freeze, but they soon disappear; they do not have the cold-hardiness of overwintering juveniles.

A simple, recently performed experiment illustrates this basic difference. This account serves, at the same time, to describe a particularly significant experiment in a series with similar results: On November 7, 1966, when the air temperature was about 15.5° C (60° Fahr.), late surviving adults of four summer species and juveniles of four overwintering species were collected on the dry, southeast slope of a grassy mesa (elevation 5,800 feet) near Boulder. (This was the last of several warm days following a cold period in which the air temperature had dropped to about − 6.6° C (20° Fahr.)). The adults were: *Hypochlora alba* (Dodge) (1 female); *Melanoplus keeleri luridus* (Dodge) (2 males, 3 females); *Phoetaliotes nebrascensis* (Thomas) (1 male, 1 female); and *Encoptolophus sordidus costalis* (Scudder) (1 female). The juveniles were: *Arphia conspersa* (4 fourth instar, 16 fifth instar); *Eritettix simplex* (11, all fourth instar); *Chortophaga viridifasciata* (1 fourth instar); and *Psoloessa delicatula* (1 third instar). These were all placed, the same day, in a low temperature cabinet (see details below) with its minimum temperature − 7.5° C for approximately 24 hours. All survived, adults as well as juveniles. The temperature was then lowered to − 12.5° and the experi-

ment repeated with the same animals (Nov. 8–9). This time the adults were all killed but all nymphs survived. Next, the temperature was lowered to − 15.5° and the juveniles were exposed 48 hours (Nov. 9–11). Five juvenile *Arphia* succumbed (four in the fourth instar and one fifth) but all other nymphs (28) survived. Finally, the survivors were exposed 48 hours to − 17.5° (Nov. 13–14). This time only four specimens died (all *Eritettix*).

According to Salt (1961), this low-temperature tolerance may be due to (1) avoidance of freezing by depression of the freezing point and the supercooling temperature or (2) ability to survive freezing or, of course, (3) a combination of these.

My study was designed to determine which alternative provides cold-hardiness in overwintering juvenile grasshoppers. It involved an analysis of survival at various low temperatures and for different periods of time, but it also included the monitoring of internal temperatures during numerous experiments. Only by measuring internal temperatures during cooling is it possible to determine supercooling limits and freezing points. Such monitoring has also demonstrated the fact that reduction in supercooling and freezing temperatures is not significantly greater in overwintering nymphs than in adults of summer species. The nymphs do survive freezing.

Monitoring internal temperatures has also demonstrated the fact that short exposures to low temperatures are not significant. Exposure must be for nearly an hour for the internal temperature of even a juvenile grasshopper to reach a low ambient temperature. The possible significance of rate of cooling in relation to tissue damage has frequently been emphasized. The present study, however, is an interpretation of an ecological situation. The rapid rates of cooling achieved by the cryobiologist, with cells and unicellular organisms (Doebbler and Cowley, 1964), do not correspond with rates limited by the slower heat exchange of organisms as large as grasshoppers.

The low temperatures used were achieved in a Cole-Parmer Low Temperature Cabinet, No. 3840. This is large (6 cu. ft.), so it is not surprising that the temperature fluctuates—about

3° C in the bottom of the cabinet—between a high, when the thermostat turns on, and a low, reached a few minutes after the compressor shuts off. The warming trend is slow, however, so low temperatures persist a long time. Since the lowest temperatures are of most significance in these studies I have indicated the temperature for a given experiment as the lowest of a given range. The range of fluctuation was reduced to about 1° in numerous experiments by using an insulated box inside the cabinet, but yielded no significant difference in results.

Temperatures were monitored with a Yellow Springs multiple-jack Tele-Thermometer, No. 44TZ. We measured ambient (cabinet) temperatures with a Yellow Springs General Purpose thermistor probe, No. 401, adjacent to the specimens; and we followed internal temperatures in an individual grasshopper with a Yellow Springs probe, No. 514. This is a 22-gauge, stainless steel, hypodermic needle in which a thermistor bead is mounted about an eighth inch back of the tip. (Only *Arphia* and *Xanthippus* juveniles could be used; *Eritettix* is too small.) These probes of this type were individually calibrated, and readings made with them were corrected accordingly.

The hypodermic was inserted through the left tympanic membrane and pushed diagonally into the head capsule along the left side so that the thermistor unit came to lie in the thorax to the left of the crop. The specimen was somewhat immobilized by being pushed headfirst into a vial only slightly larger and longer than itself. The pressure of the needle kept it from backing out. In some cases, undoubtedly, damage to the specimen resulted from unsuccessful use of the technique, but this was not inherent in the method; many individuals so treated survived long afterward.

All specimens in an experiment (that with the probe and, often, many others being tested at the same time) were placed in the cabinet simultaneously. Internal temperatures were read at one minute intervals. The temperature dropped rapidly to the supercooling temperature, then rose suddenly to the freezing point, remained constant during the freezing process, then slowly dropped to the ambient temperature (Fig. 1).

My studies were begun in the fall of 1964. During the winter of 1964–1965, Mrs. Elizabeth W. Frank, an N.S.F. Fellow, carried out experiments in cooperation with me, her observations appearing in her Master's thesis (Frank, 1965). She determined supercooling and freezing temperatures of *Arphia* and *Xanthippus* nymphs, and carried out experiments to test survival at low temperatures on these and *Eritettix*. The lowest supercooling (and freezing) temperatures she found were $-9.4°$ C (freezing at $-2.2°$) and $-9.2°$ (freezing at $-3.2°$) in *Xanthippus,* and $-9.2°$ (freezing at $-2.9°$) in Arphia. Of 16 specimens no others supercooled below $-7.4°$. Her survival tests were of short duration, but one, her longest, is of special interest: Only one *Eritettix* of a group

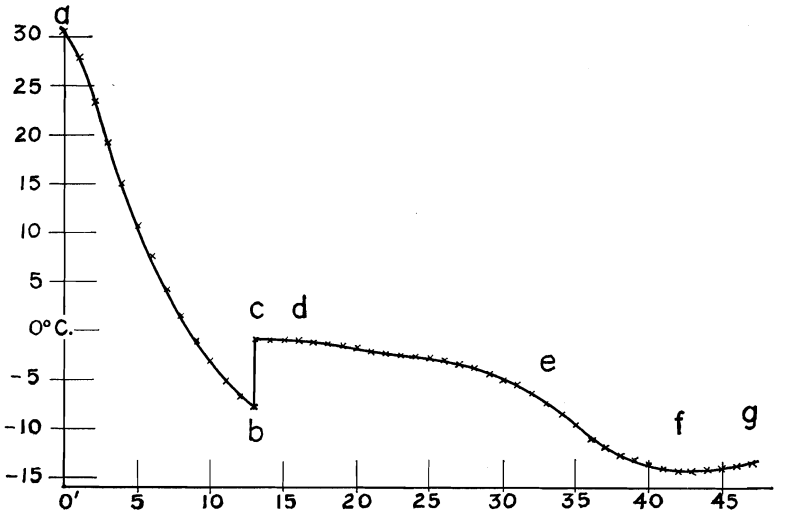

FIG. 1. Internal temperature of a fifth instar *Arphia conspersa* nymph exposed to a temperature of approximately $-15°$ C. Vertical axis, temperature C; horizontal axis, time in minutes. Explanation of letters: a, placed in cabinet; b, supercooled temperature ($-7.8°$); c, freezing point ($-1.0°$); d, end of freezing period; e, reaches supercooled temperature a second time; f, reaches ambient temperature (begins to follow temperature cycle of low temperature cabinet); g, removed from cabinet. This specimen, as well as other nymphs of *Arphia, Xanthippus,* and *Eritettix* in the same experiment, survived.

of three juvenile *Arphia* and seven juvenile *Eritettix* failed to survive an eight hour exposure to − 19° C. Of particular note in her thesis is the demonstration by her husband, Dr. William Frank, by infrared spectrum analysis, of the probable presence of glycerol in fluid obtained from *Arphia* and *Eritettix* juveniles.

During the winter of 1965–1966, with the assistance of Mrs. Judith Bodenham, I performed over 60 additional experiments along the same line. Most of these were carried out on laboratory reared specimens, juveniles and adults, of several species that normally overwinter in the egg stage, but 21 dealt with the three common species of overwintering juveniles. The design and execution of these experiments were influenced by the earlier ones; they were aimed at determining whether nymphs survive through depression of supercooling and freezing temperatures or by tolerance of freezing. As previously stated, nymphs do survive freezing. Glycerol is probably present but not in sufficient quantity to depress the freezing and supercooling points significantly. It may, however, be an important factor in preventing injury during freezing (Salt, 1961; Doebbler and Cowley, 1964).

Many of my experiments involved long exposures at low temperatures, types of tests not previously run but illustrated by the larger adult *Xanthippus* is less than half as rapid. (These are not rapid rates by physiological standards but are more rapid experiment already described. Eleven involved exposures of one to five days to temperatures below supercooling levels. Internal temperatures were not measured in these because shorter experiments, with internal monitoring, provided us with information on the rate of cooling, the time required to reach the supercooling point and to complete freezing, and the time required for a frozen grasshopper then to be cooled to the ambient temperature of the cabinet. Figure 1 shows that the rate of cooling to supercooling, when a rather small grasshopper is exposed to an ambient temperature a few degrees below its supercooling point, is nearly 3° C per minute. The rate of cooling of the much than most "natural" rates.) The ambient temperature (about 6° below supercooling) was reached by the *Arphia* nymph 30 minutes after onset of freezing. An adult *Xanthippus* reaches

such an ambient temperature about 45 minutes after onset of freezing. Thus, laboratory exposure to low temperature should be at least 60 to 90 minutes; otherwise one cannot be sure that the temperature of the specimen has dropped to that of its surroundings.

Experiments with adults of summer species show no significant differences in supercooling and freezing temperatures between them and overwintering juveniles. The difference is in ability to tolerate freezing. A few examples: Adult female *Arphia pseudonietana* (Thomas), field collected, supercooled Sept. 6, 1964, to − 8.8° C (freezing at − 1.8°), exposed to − 11° for 25 minutes after completion of freezing, no recovery; adult female *Melanoplus bivittatus* (Say), laboratory reared, supercooled Oct. 25, 1965, to − 7.5° (freezing at − 1.0°), exposed to − 14° for 10 minutes after completion of freezing, showed some breathing movements but died same day; adult male *Melanoplus femur-rubrum* (DeGeer), laboratory reared, supercooled Dec. 11, 1965 to − 7.0° (freezing at − 0.8°), exposed to − 15° for 20 minutes after completion of freezing, no recovery.

In contrast are numerous cases of survival of overwintering nymphs exposed to such temperatures not merely for a few minutes but for several days. An experiment demonstrating such survival has already been described. Four experiments involved exposures of five days each. Juvenile *Arphia* and *Xanthippus* completely recovered after five days at − 16° C, and 4 of 8 *Eritettix* juveniles survived five days at − 14°.

Such survival, to be ecologically adaptive, must of course occur repeatedly, during each period above freezing that has followed very low temperatures. Several individual specimens have illustrated this adaptation. A juvenile *Arphia* collected Oct. 28, 1965, was monitored with an internal probe on Nov. 8. It supercooled to − 8.0° C, froze at − 1.5°, and was removed to room temperature as soon as frozen. On Nov. 20–21 it survived over 50 hours at − 11.5°. It survived 24 hours exposure to − 15° on Nov. 24–25, and, after an interval at room temperature, survived five days (Dec. 12–17) at − 14°. A similar sequence can be described for a *Xanthippus* juvenile, which was

exposed 20 hours (Nov. 8) at − 15.5°, was frozen briefly Nov. 13, and subsequently survived exposure five days (Nov. 17–22) at − 12.5°.

One can be assured, by following internal temperature changes, that he is dealing with ice formation; but familiar evidence for freezing comes, too, from visual examination of frozen grasshoppers. A frozen grasshopper, whether an overwintering juvenile or a nonwintering adult, is stiff and brittle when taken from the freezing cabinet. It can be broken into parts between the fingers—the abdomen into several parts, for example. Each such break extends all the way through, the gut breaking across at the same level as the integument. When a frozen grasshopper is struck with a hammer the integument breaks primarily (though not exclusively) along existing sutures, and internal organs tend to break across. If a frozen grasshopper, thus broken or crushed, is placed quickly under a dissecting microscope one can watch the thawing. On shiny, exposed organ surfaces (where the appearance suggests ice), free liquid, presumably water, appears within one or two minutes under the influence of the heat of the microscope lamp.

These observations suggest that there is no observable difference between overwintering juveniles and nonwintering adults in the frozen condition. But overwintering juveniles survive freezing, as numerous experiments demonstrate, while adults of species that overwinter in the egg stage apparently do not.

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