NOTE TO USERS

OXYGEN CONSUMPTION AT VARIOUS TEMPERATURES BY NYMPHS AND ADULTS OF THE GRASSHOPPER, MELANOPLUS DIFFERENTIALIS (THOMAS).

by

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I. INTRODUCTION

While employed by the Grasshopper Control Office of the United States Department of Agriculture, Bureau of Entomology and Plant Quarantine, the writer accompanied Mr. Louis A. Spain of the Grasshopper Control Office on a routine visit, in the Spring of 1939, to a small egg bed of Dissosteira longipennis (Thomas), a migratory grasshopper, near Tucumcari, New Mexico, and had occasion to observe the interesting extinction of this small infestation. Mr. Spain had followed the development of the infestation from time of hatching and among other notes had records of the population density occurring at each visit. On reaching the designated area Mr. Spain and the writer were surprised to find no sign of the expected infestation of third instar nymphs. After scouting over the area for some time, dead nymphs were found; counts on the ground checked reasonably well with the expected population.

Since the infestation was a small one, it was being used for purposes of observation. Baiting crews were questioned, and it was ascertained that in accordance with previous instructions no bait had been spread on the area. Thus, a minor mystery arose regarding the nature of the death of these grasshoppers.

Observations had been started on the minimum temperature at which Dissosteira longipennis began its feeding activities. The daily weather reports had been followed for this and other areas since the beginning of the study, and the routine checking of this information showed that during the week previous to the visit the highest daytime temperature had been several degrees below what seemed to be the feeding temperature for D. longipennis; yet the lowest daytime temperature had not been more than 10 degrees Fahr. below the feeding temperature. In subsequent discussions concerning the observation, the question arose as to whether the grasshoppers were unable to feed and had starved to death in the interval between visits.

On returning to Iowa State College in the Fall of 1939 the writer decided to make some determinations of the rate of respiratory metabolism of grasshoppers at various temperatures. It was hoped that the results from such experiments might contribute to the understanding of field observations such as the one mentioned above.

Even though D. longipennis has been reared in the laboratory, techniques and food habits are not completely worked out. For this reason it seemed expedient to use, in the initial observations of respiratory metabolism, a grasshopper occurring in Iowa and one which could be reared in the laboratory fairly easily. Melanoplus differentialis (Thomas) met these requirements. Since M. differentialis

is a pest of great economic importance, work which contributes to the general understanding of the insect and its habits may have an associated useful and practical value. The work of Parker (1929), Parker and Shotwell (1932) and Shotwell (1941) offers the results of detailed observations, both in the field and in the laboratory, of the feeding and other behavior responses of M. differentialis to changing temperatures. The author, while engaged in field work in the control of this and other grasshoppers, had opportunity to verify these observations and gain, at first hand, some experience in the habits of this insect under field conditions.

II. HISTORICAL

The invention of the manometric respirometer by August Krogh in 1914 provided an instrument sufficiently sensitive to give accurate results in the study of the respiratory metabolism of insects. However, this important turning point in the study of insect metabolism occurred over one hundred years after the earliest interest in the subject.

A. Review of Literature

Introduction

According to Sayle (1928a) Spallanzani in 1807 made a number of observations on the respiratory exchanges of larvae, chrysalides and adults of silkworms, flies, bees and wasps; and Treviranus in 1831 studied the gaseous exchange of various animals including some insects such as butterflies, moths, flies and beetles. Treviranus calculated the oxygen consumption per kilogram per hour for one of the beetles at a temperature of 13° - 15° Cent., and also measured the oxygen consumption and carbon dioxide output of three bees at 11.5° Cent. Treviranus observed that the honey bee utilized more oxygen at 27.5° Cent. than at 14° Cent.

Newport (1836) wrote in the introduction to his paper: ".....it has been long proved by many physiologists that

insects produce the same changes in the atmosphere during respiration as other animals" namely, ".....the production of carbonic acid gas and loss of oxygen". He studied the gaseous exchange of nine species of insects, each in various stages of development.

A study of the effect of temperature on the gaseous exchange of the cockroach, Blatta orientalis, was made by Butschli (1874) who found that a rise in temperature was followed by a regular rise in rate of metabolism. He observed that the gaseous exchange at 15° Cent. was twice as great as at 4° Cent., and at 32° Cent. it was seventeen times greater than at 3° Cent. Butschli also studied the effect of starvation on the rate of metabolism and found a decrease in rate with each 24 hour period of starvation.

Bert (1885) determined the oxygen uptake and carbon dioxide output for larval, pupal and adult stages of Bombyx mori. Using a method of air analysis patterned after the Haldane method, Vernon (1897) measured the carbon dioxide output of Blatta orientalis at intervals of 3° Cent. between 30° Cent. and 2° Cent. He found that as the temperature was lowered the output of carbon dioxide diminished, and, as the temperature was raised, it increased.

Parhon (1909) made an interesting observation in studying the effect of temperature on the rate of metabolism in the honeybee. Parhon found that with flies there were successive increases in oxygen consumption in cc./kg./hr. with

increases in temperature over a range from 0° Cent. to 37° Cent. With the honeybee, however, there were increases in oxygen consumption, in cc./kg./hr. with increases in temperature from 0° Cent. to 10° Cent., but decreases in oxygen consumption with increases in temperature from 10° Cent. to 20° Cent., and further decreases in oxygen consumption with increases in temperature up to 45° Cent. She concluded that bees differ from most insects and resemble homoiothermic animals in attempting to regulate their body temperature by changes in rate of metabolism in response to fluctuations in the temperature of their surroundings. Her results show that bees resist cold by an increase in metabolic rate, thereby producing heat, and by a retention of water in the body tissues, thereby saving the heat which would be lost by evaporation, and that bees resist heat by the opposite combination: a decrease in metabolic rate and an elimination of water through the respiratory surfaces. It is a well established observation that bees ventilate the hive by fanning in warm weather and conserve heat by clustering in cold weather.

Krogh (1914) devised a manometric respirometer which he called "ein Mikrorespirationsapparat" and used it to study the effect of temperature on the respiratory metabolism of Tenebrio molitor pupae and other insects. This instrument is sufficiently sensitive to give accurate results in the study of insect metabolism, and it, or some modifica-

tion of it, has been widely used since its invention.

Differences in the respiratory metabolism among insect species.

When one takes into account the variations in the conditions under which members of the class Insecta exist, the great differences in size, shape, nature of exoskeleton, external and internal anatomy, environment, food habits, metamorphosis, and many other considerations in their nature and mode of existence, it is only reasonable to expect differences in the respiratory metabolism of the many species. Differences in the rate of metabolism per unit of body weight per unit of time at a given temperature and differences in the respiratory quotient are of chief importance. Bodine (1921) found differences in the rate of carbon dioxide output for certain species of Orthoptera; many other illustrations of insect respiratory metabolism may be found in Table 1.

While the response in rate of metabolism to certain factors, such as changes in temperature, seems to be generally similar, exceptions have been noted. This should be kept in mind in applying generalizations regarding the respiratory metabolism of insects to a particular species.

Effect of temperature on the rate of metabolism of insects.

Most insects are poikilothermic in their response to changes in temperature. Increases in temperature, starting

with a low temperature, are accompanied by an increased rate of metabolism up to a maximum where further increases in temperature result in a decreasing rate of metabolism until death. Falling temperature is generally accompanied by a decreasing rate of metabolism.

Support for the generalizations above is found in the works of Butschli (1874) and Vernon (1897) on the cockroach, Blatta orientalis, of Bodine (1921) on certain Orthoptera (grasshoppers), of Dirken (1922) with the cockroach, Periplaneta americana, and of Buddenbrock and Rohr (1923) with the walking stick, Dixippus morosus. Rogers (1929), in working with the grasshopper, Melanoplus differentialis, found an increased rate of oxygen consumption with successive increases in temperature.

The results of the following workers, listed in Table 1, also show an increased rate of metabolism with increases in temperature: Batelli and Stern (1913) working with adults of Melolontha vulgaris, larvae and adults of Musca vomitoria and larvae and adults of Bombyx mori; Ellinger (1916) on adults of the mosquito, Culex annulatus; Cook (1927) on larvae of the cutworm, Chorizagrotis auxiliaris; Sayle (1928) using nymphs of the dragon fly, Aeschna umbrosa; Dreyer (1932) with adults of an ant, Formica ulkei; Kleinman (1934) working with nymphs of the grasshopper, Chortophaga viridifasciata, and larvae of the Japanese beetle, Popillia japonica; Argo (1939) with adults of the cluster fly, Pollenia rudis, and of the citrus mealy-

bug, Pseudococcus citri, and with eggs of the milkweed bug, Oncapeltus fasciatus; and Rathjen (1939) with larvae of the caddis fly, Enoicyla pusilla.

As previously stated on page 6, Parhon (1909) found that in certain respects the honeybee is an exception to the general rule for insects that increases in temperature are accompanied by an increased rate of metabolism. Kosmin et al. (1932) also found the honeybee different from other cold blooded animals, and more like homoiothermic animals in its reaction to sinking temperature and concluded that since the bee is a social animal it makes an attempt to regulate the temperature of the colony.

Relationship of size to rate of metabolism.

Rubner's surface law. Rubner (1883), working with various warm blooded animals, showed that the rate of metabolism is proportional to the body surface, i.e., smaller animals, in relation to their body weight, have a higher metabolic rate than larger animals. This is sometimes referred to as Rubner's Surface Law and holds that for like but different larger animals the intensity of metabolism is proportional to the surface and not to the volume. Bock (1930) proposed that the surface law of Rubner, known to be true for vertebrates and for many invertebrates, was also applicable to insects.

The application of Rubner's surface law to insects. Bodine (1921) stated that lighter and younger animals have higher rates of carbon dioxide output and thus indicated the possible relation of a surface law holding for grasshoppers. The conclusion that the surface law of Rubner is true for the locust, Locusta migratoria, was reached by Butler et al. (1936). Working with the grasshopper, Chortophaga viridifasciata, Ludwig (1937) found no significant difference in the metabolic rate of nymphs and adults of the same body weight.

Referring to Bock (1930), and Melampy and Willis (1939) of Table 1, a decrease in rate of metabolism with an increase in size is shown for larvae of Cloeon dipterum (Ephemerida); nymphs of Aeschna umbrosa (Odonata); larvae of Tenebrio molitor (Coleoptera); larvae of Phlegethontius sexta (Lepidoptera); and larvae of Apis mellifica (Hymenoptera).

The threshold temperature for spontaneous movement.

As insects are cold blooded, their metabolism and activity are influenced by the temperature of their bodies which, in turn, depends largely on the temperature of the surrounding environment. Low temperature inhibits activity, and a higher temperature usually stimulates. Insects can survive over a range limited above and below by lethal temperatures, but the range of normal activity is generally much narrower than the outside limits.

Distinction between chill-coma temperature and the

threshold temperature for spontaneous movement. Belehradek (1935) defined the "chill-coma" temperature as the temperature at which the insect is immobilized by cold, and the "cold-death point" as the temperature below which exposure is lethal. Mellanby (1939) points out that most previous workers have made the mistake of assuming that an insect under natural conditions will spontaneously exhibit activity until the temperature falls to that at which "chill-coma" occurs. He is of the opinion that this is not necessarily the case and states that in his experience the lowest temperature at which some form of activity is possible frequently appears to be several degrees below that at which spontaneous movements take place. In experimental work with the tsetse fly, Glossina palpalis, Mellanby found that the insect never flew to seek food below 20° Cent., though it was able to fly at 14° Cent., and observations made in the field showed that the insects never appeared until the temperature among the bush in which they were lurking rose above 20° Cent.

The term "threshold temperature for spontaneous movement" has been coined by Mellanby and defined as the minimum temperature in an insect's range at which spontaneous movements occur. Mellanby (1939, page 473) makes this important observation:

> "It appears that if there is one single factor more than any other which controls the distribution of an insect, it is the temperature below which activity never normally takes place. Insect distribution

> and survival are no doubt greatly affected by such factors as lethal high and low temperatures and unfavourable atmospheric humidity, but if, in any region, the temperature does not rise sufficiently often above that at which the normal activity of a species begins, that species will cease to exist although all other conditions are favourable to life."

Mellanby's experiments indicate that while the "chill-coma" temperature varies with previous treatment, the threshold temperature for spontaneous movement will be the most constantly fixed point in the lower range of an insect's experience.

The threshold temperature for spontaneous movement of Melanoplus differentialis. Determination of the threshold temperature for spontaneous movement can be done best by field observations of the insect in its natural habitat. Dr. J. R. Parker and Mr. R. L. Shotwell of the U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine, have carried out extensive investigations of the habits and control of the grasshoppers of economic importance in the Great Plains area of the United States. Parker and Shotwell (1932) reported field observations on the movements and feeding of nymphs and adults of Melanoplus differentialis and Melanoplus bivittatus. Shotwell (1941) summarized the results of observations, made at intervals over a period of 10 years, on the relationship of temperature to the activities and feeding of nymphs and adults of M. differentialis and M. bivittatus. Nymphs and adults of these species roost

on plants above the surface of the soil during the night. In the morning when the air temperature reaches 68° Fahr. or goes above, and sunlight strikes the plants and the surface of the ground, the grasshoppers become active and begin to feed. In the field observations of both Parker and Shotwell the lowest temperature at which M. differentialis became active voluntarily and started to feed was 68° Fahr. In tests on bait materials, grasshoppers roosting on the bait pans were observed to nibble at the bait when the air temperature was as low as 55° Fahr., but Shotwell states this is unusual and lists 68° Fahr. as the temperature at which general activity and feeding began. Thus, it appears from the work of Parker and Shotwell that the threshold temperature for spontaneous movement in M. differentialis is 68° Fahr.

Relationship of the threshold temperature for spontaneous movement to starvation in grasshoppers. Parker (1929) found that nymphs and adults of Melanoplus atlantis held at constant temperatures of 8° Cent. (46.4° Fahr.) and 12° Cent. (53.6° Fahr.) did not feed, and lived for the same time at both temperatures; the nymphs lived seventeen days and the adults twenty-two days. Both nymphs and adults fed slightly at 16° Cent. (60.8° Fahr.) and died sooner, the nymphs in eleven days and the adults in twelve days. The results of Parker's experiments showed that in the narrow zone where temperature was barely high enough to bring about slight feeding and acti-

vity, death always occurred sooner than at low temperatures at which there was no feeding or at higher temperatures where normal activities and feeding were taking place. Parker suggests this as a possible explanation of the high death rate among young grasshoppers hatched by unseasonable warm weather early in the spring, followed usually by cool spells during which the young 'hoppers are kept for days on the border line of inactivity.

Variations in rate of metabolism between sexes.

Rogers (1929) reported no significant difference in the rate of oxygen consumption per unit weight per unit time of male and female nymphs of Melanoplus differentialis. Working with Locusta migratoria, Butler et al. (1936) found a slightly higher rate of metabolism per unit weight per unit time for males of any instar than for corresponding females. A higher metabolic rate per unit weight per unit time for adult males of Chortophaga viridifasciata than for the adult females was shown by Ludwig (1937).

Effect of increasing age on the rate of respiratory metabolism of adult insects.

Fluctuations in the oxygen uptake per gram of body weight per hour were found by Eddy (1931) for adults of Drosophila melanogaster, but no general trend of increase or decrease in rate with increase in age seems apparent.

In the results of Vinberg (1937), on the oxygen consumption per gram of body weight per hour of adult Drosophila melanogaster, there is a suggestion of a slightly higher rate during the approximate middle of adult life with about the same rates for the beginning and end. The work of Melampy and Willis (1939) on the respiratory metabolism of the adult worker honeybee shows a somewhat "U"-shaped curve for rate of metabolism during adult life; from the initial high point the rate decreased to a minimum at seven to eight days of age then increased steadily until death.

Effect of starvation on the rate of metabolism.

Butschli (1874) found a decrease in rate of metabolism of Blatta orientalis with each twenty-four hour period of starvation. Bodine (1921) observed that starvation caused a decrease in the rate of carbon dioxide outout of certain grasshoppers and that after the starved insects had fed there was an increase in their carbon dioxide output. According to Sayle (1928) the carbon dioxide output of Aeschna umbrosa larvae decreased with starvation except during the second week. The reason for the rise in rate of metabolism during the second week was not apparent. Tauchert (1939) showed that the oxygen uptake of the mealworm, May beetle and larva of Vanessa urticae decreased rapidly during the first twenty-four hours of starvation and in the case of the mealworm and the May beetle remained at a low level from twenty-four to

forty-eight hours of starvation. Observations on the respiratory metabolism of Formica ulkei by Dreyer (1932) showed a reduced rate during both hibernation and starvation. In hibernation, activity and rate of metabolism were uniformly lowered from the beginning; in starvation the animal remained active, depleting its reserves, and the metabolic rate declined progressively as this occurred. The ultimate result was a greater depletion than occurred in hibernation, and finally death. It was concluded by Ludwig (1937) that a decrease in oxygen consumption and respiratory quotient of the grasshopper, Chortophaga viridifasciata, was the result of starvation. Bellucci (1939) found that starvation caused the metabolic rate of Japanese beetle larvae to fall rapidly during the first two days, then more slowly to a low level which was maintained until death.

Respiratory metabolism of insect eggs and pupae.

Eggs. The rate of oxygen uptake of an insect egg is dependent on its type of development. In those insect eggs that have a continuous development from time of laying until hatching, the oxygen uptake shows a steady increase throughout the developmental period. Insect eggs which have a diapause show a fairly high oxygen uptake at the beginning of development, a decrease to a low level during diapause and a sharp rise with renewed development at the end of diapause.

Pupae. Insect pupae were chosen by many early investi-

gators of insect metabolism to minimize the factor of movement while the insect was in the manometric flask. They generally overlooked the possibility of different rates of metabolism with various phases of pupal development. Later work, notably that of Taylor (1927) and Taylor and Steinbach (1931), has shown that the rate of metabolism during the pupal development of insects exhibits a somewhat "U"-shaped curve decreasing from the initial value to a minimum just preceding the time of emergence. The sharp upswing at this time reaching an extremely high rate of metabolism as compared to that at any other time during pupal life is undoubtedly due to the strong body movements accompanying eclosion.

Table 1. Summary of Measurements of the Respiratory Metabolism of Insects for the Period 1914 through 1941.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R.Q.
ORTHOPTERA							
Blatta	Hiestand (1932)	Titration of $Ba(OH)_2$	adult	22		1.391	
Blatella germanica	Gunn (1935)	Barcroft	adult	30	.780		
Blatta orientalis	Gunn (1935)	Barcroft	adult	30	.518		
Chortophaga viridifasciata	Kleinman (1934)	Krogh micro-respirometer	active nymphs	7	.0846	.0516	.605
				15	.338	.218	.653
				20	.422	.306	.714
				25	.683	.527	.766
				29	.817	.673	.827
				34	1.176	.932	.795
			hibernating nymphs	15	.312	.194	.625
				27	.622	.516	.830
Chortophaga viridifasciata	Ludwig (1937)	Krogh	adult	25	.772	.655	.847
Crytocercus punctulatus	Gilmour (1940)	Warburg	adult	5	.028		
				7.5	.046		

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R.Q.
ORTHOPTERA							
Locusta migratoria	Butler et al. (1936)	Barcroft	Migratory adult ♀	27	.776		
			Solitary adult ♂		.779		
			Migratory 3 rd ♀		.848		
			Solitary 1 st ♂		3.738		
Melanoplus	Hiestand (1932)	Titration of $Ba(OH)_2$	adult	22		.937 .648	
Microcentrum	" "	" " "	"	22		.382	
Periplaneta	" "	" " "	"	22		.577	
Periplaneta americana	Gunn (1935)	Barcroft	adult	30	.421		
Periplaneta americana	Perfentjev & Devrient (1930)	Warburg	adult	26	.63	.47	.75

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R.Q.
ISOPTERA							
Termopsis nevadensis	Cook (1932)	Warburg	nymph	20	.433		
EPHEMERIDA							
Baetis sp.	Fox et al. (1937)	Winkler water anal.	larval	10	4.000		
B. rhodani	Fox & Simmonds (1933)	Barcroft	nymph	10	2.571		
				16	5.619		
				16	4.483		
Baetis scambus	Fox et al. (1937)	Winkler water anal.	larval	10	1.870		
Cloeon dipterum	"	"	larval	10	1.310		
Cloeon dipterum	Fox & Simmonds (1933)	Barcroft	larval	10	0.600		
			larval	10	1.792		
Cloeon dipterum	Hernisch (1939)	Warburg	larval	20	.793		
			8/10/39		.793		
			8/11/39		.496		
			8/17/39		.431		
			8/24/39		.344		
			8/29/39		.389		

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R.Q.
EPHEMERIDA							
Ecdyomurus venosus	Fox et al. (1935)	Barcroft	nymph	10	1.321		
Ephemera damicia	"	"	nymph	10	.829		
Ephemera vulgata	"	"	nymph	10	.740		
Ephemera vulgata	Harnisch (1939)	Warburg	larval	20	.118		
Leptophlebia marginata	Fox et al. (1937)	Winkler water anal.	larval	10	1.390		
L. vespertina	"	"	larval	10	2.130		
ODONATA							
Aeschna umbrosa	Sayle (1928)	Titration of $Ba(OH)_2$	larval	3		.0275	
				9		.060	
				13		.1427	
				17		.177	
				22		.276	
				25		.324	
				28		.343	
				31		.344	
				34		.317	

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R.Q.
ODONATA							
Aeschna umbrosa	Sayle (1928)	Titration of $Ba(OH)_2$	nymphs weighing .167 mg	22			.377
			.185 "				.367
			.216 "				.355
			.238 "				.330
			.264 "				.293
			.287 "				.242
			.315 "				.178
			.342 "				.148
TRICHOPTERA							
Enoicyla pusilla	Rathjen (1939)	Winkler water anal.	larval	4	.529		
				10.8	.554		
				15.5	.661		
				17.8	.773		
				20.2	.952		
				22.9	1.097		
				29.1	1.191		
				32.7	0.973		
Hydropsyche sp.	Fox & Simmonds (1933)	Barcroft	larval	15	.769		
Molanna sp.	"	"	larval	15	.511		

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R.Q.
HOMOPTERA							
Pseudococcus citri	Argo (1939)	Thunberg micro-respirometer	adult	2.2	.120		
				7.1	.222		
				12.2	.384		
				17.3	.702		
				21.9	1.08		
				26.3	1.764		
				30.9	2.172		
				35.1	2.238		
				40.1	2.178		
				45.2	2.754		
				47.6	3.51		
				48.5	1.014		
COLEOPTERA							
Coccinella	Hiestand (1932)	Titration of $Ba(OH)_2$	adult	22		.208	
Leptinotarsa decemlineata	Fink (1925)	Krogh		21-24		1.5	
Melolontha vulgaris	Batelli & Stern (1913)	air analysis		20	.930	.610	.65
				30	1.620	1.120	.69
				40	3.030	2.190	.72
				45	4.400	3.200	.73
				50	4.250	3.500	.82

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R.Q.
COLEOPTERA							
Popilla japonica	Kleinman (1934)	Krogh micro-respirometer	third	10	.0918	.0642	.681
				15	.205	.145	.693
				20	.357	.262	.739
				25	.413	.273	.782
				34	.815	.715	.879
Popilla japonica	Bellucci (1939)	Krogh		25	.393		
Tenebrio molitor	Schmalfusz et al.(1939)	Krogh	adult	19.6	.375		
		Krogh		22.5	.612		
Tenebrio molitor	Michal (1931)	manometric set-up devised by Michal	larva weighing	33			
			0.5 mg		3.66		
			1. "		3.66		
			2. "		3.25		
			5. "		3.38		
			9. "		3.41		
			13. "		2.88		
			15. "		2.66		
			27. "		1.32		
			29. "		1.06		
			46. "		1.10		
			62. "		0.91		
			75. "		0.75		

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R. Q.
COLEOPTERA							
Tenebrio molitor continued	Michal(1931) continued	manometric set-up devised by Michal	larva weighing 95.mg	33	0.56		
			108. "		0.73		
			130. "		0.56		
			139. "		0.67		
Tetraopes	Hiestand (1932)	Titration of $Ba(OH)_2$	adult	22		.121	
Tribolium confusum	Park (1936)	Krogh	adult ♂	28	1.79		
			adult ♀		2.01		
DIPTERA							
Calliphora erythrocephala	Fraenkel & Hereford (1938)	Barcroft	larval	17	.53	.37	.70
				27	.90	.57	.64
Chironomus	Hiestand (1932)	Titration of $Ba(OH)_2$	adult	22		1.067	
Chironomus thummi	Harnisch (1936)	Warburg	larval	19	.105		
				20	.140		
				21	.170		
				21.5	.126		
				22	.144		

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R.Q.
DIPTERA							
Culex annulatus	Ellinger (1916)	Krogh micro-respirom.	over-winter-ing adults	3.2	.099		
				11	.229		
				20	.834		
Drosophila melanogaster	Eddy (1931)	Krogh	adult age	25			
			2 days		4.357		
			6 "		6.331		
			9 "		4.563		
			10 "		5.968		
			11 "		4.927		
			12 "		8.695		
			14 "		4.640		
			15 "		6.746		
			16 "		8.879		
			17 "		6.971		
			18 "		6.807		
Drosophila melanogaster	Kucera (1934)	Fenn micro-respirom.	adult♂	26	2.136		
			adult♀		2.584		
Drosophila repleta	Chadwick et al. (1940)	Warburg	adult	25	1.66		

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R. Q.
DIPTERA							
Drosophila melanogaster	Vinberg (1937)	Warburg	adult age	22			
			4 days		2.24		
			6 "		2.22		
			9 "		2.54		
			12 "		3.20		
			18 "		2.96		
			20 "		3.11		
			22 "		2.36		
Musca vomitoria	Batelli & Stern (1913)	air analysis	larval	20	1.30	1.05	.81
				30	2.04	1.65	.81
				40	3.90	3.28	.84
				45	4.9	4.16	.85
				50	5.2	4.52	.87
				55	4.6	4.05	.88
			adult	10	.96	.59	.61
				20	3.10	2.30	.74
				30	5.80	4.40	.76
				40	9.60	7.50	.78
				45	14.70	11.10	.76
				50	12.90	9.70	.75
Musca	Hiestand (1932)	Titration of $Ba(OH)_2$	adult	22		1.944	

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R. Q.
DIPTERA							
Pollenia rudis	Argo (1939)	Fenn micro-respirometer	adult	- 1.8	.048		
				.9	.072		
				4.9	.168		
				9.9	.312		
				14.9	.612		
				19.7	.852		
				24.5	1.104		
				30	1.422		
				33.8	1.944		
				37.6	3.702		
LEPIDOPTERA							
Bombyx mori	Batelli & Stern (1913)	air analysis	larval	20	.68	.62	.91
				30	.84	.78	.93
				40	1.100	.95	.86
			adult	20	1.400	.900	.64
				30	2.700	1.800	.67
				40	6.200	4.28	.69
Chorizagrotis auxiliaris	Cook (1927)	Titration of $Ba(CH)_2$	larval	8		.145	
				12		.189	
				22		.378	
				27		.422	
				32		.426	
				37		.442	

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R. Q.
LEPIDOPTERA							
Deilephila elpenor	Kalmus (1929)	gas analysis	adult	20-22		.180	
HYMENOPTERA							
Apis mellifica	Bock (1930)	Warburg	young round larva	25	.952	.780	.82
			same		1.143	.875	.76
			medium round larva		.925	.620	.67
			old round larva		.606	.378	.63
			extended larva		.339	.205	.60
			imago		1.317	.895	.68
			1-2 day adult		2.29	1.76	.77

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc O_2 /gr/hr.	cc. CO_2 /gr/hr.	R. Q.
HYMENOPTERA							
Apis mellifica	Kosmin et al. (1932)	Barcroft Differential	Adult♀ at rest	11	.024		
			adult♀ crawling slowly		1.20		
			adult♀ at rest	18	.054		
			adult♀ crawling slowly		.480		
			adult♀ crawling slowly	35	.300		
Apis mellifica	Melampy & Willis (1939)	Warburg	larva♀ days old	35			
			2-3		4.554	5.208	1.14
			3-4		3.423	4.130	1.21
			4-5		2.446	3.15	1.29
			adult worker days old				
			2-3		2.901	4.121	1.42

Table 1 Cont'd.

Insect	Author	Manometric Technique	Stage	Temp. °C.	cc. O_2 /gr/hr.	cc. CO_2 /gr/hr.	R. Q.
HYMENOPTERA							
Apis mellifica continued	Melampy & Willis (1939) continued	Warburg	adult worker days old	35			
			3-4		2.238	2.746	1.23
			4-5		1.063	1.308	1.23
			5-6		.627	.710	1.13
			6-7		.409	.425	1.04
			7-8		.374	.384	1.03
			8-9		.396	.417	1.05
			9-10		.414	.401	.97
			10-11		.415	.399	.96
			11-12		.466	.445	.95
			12-13		.509	.482	.95
			13-14		.629	.593	.94
			14-15		.754	.692	.92
			15-16		.818	.759	.93
			16-17		1.025	.963	.94
Formica ulkei	Dreyer (1932)	Harrington & Crocker microrespirometer	adult	4	.309	.154	.50
				7	.306	.184	.60
				10.5	.482	.309	.64
				15.5	.568	.401	.70
				18.5	.646	.509	.78
				22	.848	.735	.86
Vespa Folistes	Hiestand (1932)	Titration of $Ba(OH)_2$	adult	22		8.67	
			adult	22		1.822	

III. MATERIALS

A. Experimental Insect

Determination of species of experimental insect.

Melanoplus differentialis (Thomas) was chosen as the experimental insect because it and its eggs are available in Iowa, and the species is easy to rear in the laboratory. It is not easy to distinguish rapidly between first instar nymphs of several species of the genus Melanoplus. To insure the use of a single species, all individuals used were hatched from eggs identified as those of M. differentialis according to the method of Tuck (1939).

Eggs.

Field collection and laboratory separation and identification. Grasshopper eggs were collected in the field in the Fall of 1940 and brought into the laboratory where they were separated and identified as mentioned above.

Hatching of the eggs. Those eggs which were used as soon after collection as possible had their diapause broken by placing them in the freezing unit of an electric refrigerator for thirty days. Eggs to be used later were placed in an outside cellar which offered both a good place for storage and temperatures low enough to break the diapause of the

eggs.

Petri dishes about 6 inches in diameter and 2½ inches deep were filled approximately one-fourth full with damp sand of roughly 10 percent water content by weight. Eggs and egg-pods were placed on the sand and covered over with sand of about the same moisture content until the eggs were approximately ½ inch below the surface. The petri dish with the eggs was placed in a constant temperature cabinet operating at 30° Cent. At this temperature the eggs hatched in about fourteen days. As they hatched, the nymphs were removed twice daily and placed in Riley cages.

Nymphs.

Type of cage used for rearing nymphs and adults. In the Riley type cages used for rearing and handling the nymphal and adult grasshoppers, the base was 12 by 12 by 6 inches and filled with soil; the cage was 12 by 12 by 24 inches. Since first instar nymphs can escape through the mesh of ordinary window screen, white bolting cloth was used on the cages in which first and second instar nymphs were kept. Three of the sides and the top were of bolting cloth; the remaining side was of glass. The glass sheet was set in grooves and could be removed by sliding it upward. Observation of the activities inside the cage could be made through the glass, and entrance into the cage could be obtained by sliding the glass sheet upward. Older nymphs were placed in cages with sides

of either bolting cloth or window screen.

Location of the cages. During the late fall, winter and early spring the cages were set up on the soil-bench at the east side of the north section of the west wing of the insectary greenhouse. This is part of the heated section and had a minimum temperature of approximately 70° Fahr. Since the walls and roof are glass, sunlight was available at all times during the day. During the late spring, summer and early fall the cages were set up outside where sunlight was available at all times during the day.

Plants used for feed. Barley, wheat, corn and alfalfa were planted in pots about 4 inches in diameter and 5 inches high of the type ordinarily used in greenhouses.

Since first and second instar nymphs do not feed readily or easily on corn, alfalfa and wheat or barley were placed in the cages used for first and second instar nymphs. For older nymphs and adults, alfalfa, corn and barley or wheat were all present in the cages offering a selection of these plants for food at all times. A supply of fresh material was kept available and placed in the cages whenever necessary. Plants in the cages were watered twice daily, but care was taken not to spray any water on the grasshoppers as this seems to produce a high mortality, particularly in first and second instar nymphs.

Separation of instars. The instars were separated according to the key for M. bivittatus devised by Shotwell (1941), and each instar was kept separate from the others. This, of

course, facilitated the ready selection of individuals for use in the respective observations on respiratory metabolism. The number of grasshoppers in any one cage was controlled to eliminate crowding.

Collection of nymphs and adults in the field. For experiments on the effect of age of adults on the rate of metabolism, a field collection was made of nymphs of M. differentialis. Adults of Brachystola magna (Scudder), the large lubber grasshopper, Schistocerca lineata Scudder, a grasshopper, and Scudderia furcata Brunner, a katydid, were also collected in the field and brought into Ames.

The nymphs and adults collected in the field and brought into Ames were placed in cages and treated the same as nymphs reared from eggs. However, it was noticed that adults of Schistocerca lineata were not feeding heavily on the selection of plants offerred them. A further selection, among them beans, was tried. The beans seemed to be satisfactory and were placed in the cages for S. lineata along with corn and alfalfa.

Adults of Periplaneta americana (Linn.), the American roach, were taken from stock cages established for several years in the laboratory at Ames.

B. Respirometer

The Warburg manometer[1], or the Constant-Volume type of respirometer, was the instrument used to measure respiratory metabolism.

Manometric flasks.

Sizes of flasks and number of 'hoppers used in each size. Three sizes, approximately 5 c.c., 15 c.c. and 150 c. c., of standard Warburg flasks with central well were used. The 5 c.c. flask accomodated ten first instar nymphs or five second instar nymphs without undue crowding. Five third instar nymphs or three fourth instar nymphs were handled easily by the 15 c.c. flask. The 150 c.c. flask was used for fifth instar nymphs and adults; five fifth instar nymohs or two adults were placed in each flask with the exception of Brachystola magna adults which were so large that the use of only one adult per flask seemed expedient. Since the movements of the grasshoppers were not restricted while in the manometer flasks, the elimination of crowding was a necessary precaution. Undue crowding by confining too many individuals in a small space excites and irritates the grasshoppers, re-

[1]The Warburg manometers, Warburg manometer flasks, Quick-set bi-metallic thermostat, relay and mercury switch for thermostat were of the standard type as supplied by the American Instrument Company, Silver Spring, Maryland.

sulting in general hopping and moving about. This unnecessary movement would bring about a great increase in metabolic rate, particularly at the higher temperatures, and the uneven nature of the increased rate would only serve to introduce error into the results. Grasshoppers under field conditions are content, for the most part, to crawl over the vegetation or surface of the ground, hopping only occasionally, unless disturbed.

Contact of grasshoppers to KOH in central well of the manometric flask prevented. Twenty percent KOH was placed in the central well of the manometer flask to take up the carbon dioxide. A small piece of filter paper carefully folded to fit the central well was placed there to afford a greater surface for the KOH. In the 5 c.c. and 15 c.c. flasks a tight fitting cap of fine mesh copper screen was made to fit over the central well to prevent the grasshoppers from crawling into the well and coming in contact with the KOH. Care was taken that no KOH was present on the copper screen.

The 150 c.c. flask was of the open type, like a beaker, and had a top which fitted on through a ground glass connection. In the top was the seat of the ground glass connection to the manometer. There were three equally spaced small glass hooks projecting inward from the side of the flask, slightly less than half-way up from the bottom. A fine mesh copper screen resting on these hooks provided a

platform for the grasshoppers and prevented their coming in contact with the KOH in the central well of the flask.

C. Water Bath

A Freas water bath of approximately fifteen gallon capacity was used; this was equipped with a pump to circulate the water within the bath.

Control of temperature in the water bath.

Provision for temperatures above room temperature. Temperature control of the water bath was accomplished through the use of a quick-set bimetallic thermostat accurate to 0.1° Cent. (0.18° Fahr.). Since several different temperatures were used, the quick-set nature of the thermostat was particularly useful. Three blade type heating elements, connected to the thermostat through a relay, provided the heating unit. The temperature of the water bath was checked after each change and during the interval the manometers were present in the bath through the use of a mercury thermometer.

Provision for temperatures below room temperature. When temperatures below that of room temperature were desired, a cooling unit was used. To make the cooling unit, 3/8 inch copper tubing was shaped into a double coil, the inner one separated from the outer by about 1 inch. The spirals of the coils were likewise separated about an inch. The double

coil was placed vertically in a glazed earthenware crock of about 20 gallons capacity and connected through rubber pressure tubing to a flat coil of copper tubing placed in the bottom of the water bath. Water coming from the pump in the bath passed directly over this coil. A one-way valve plunger type pump inserted into the circuit provided circulation of water from the coil in the crock to the coil in the water bath through the pump and back to the coil in the crock. Connections between the coils and pump were of rubber pressure tubing.

When the crock was filled with cracked ice, a temperature of 40° Fahr. could be maintained easily in the water bath. For the use of temperatures below that of room temperature, the effect of the cooling coil was balanced by the heating unit and provided a well established temperature.

IV. METHODS

A. Calculation of the Manometric Constant

Method given by Dixon.

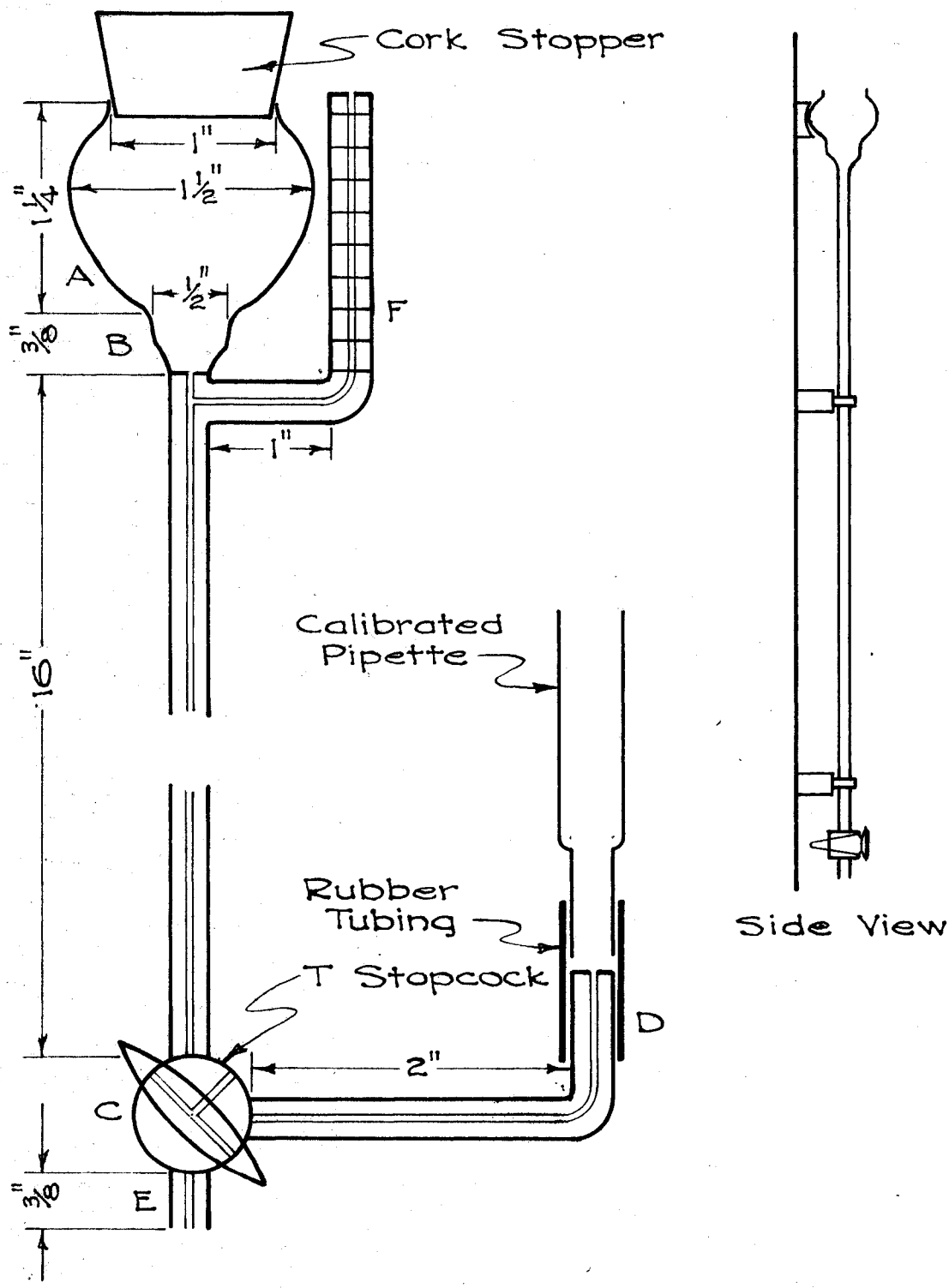
The manometers and flasks were calibrated and the manometric constant for each set of manometer and flasks was calculated according to the method given in Dixon (1934). The factor α, the solubility of the evolved gas in the liquid in the vessel (in c.mm. of gas at N.T.P.) dissolved in 1 c.mm. of liquid when in equilibrium with a partial pressure of the gas equal to the factor P_o, was not used in the calculations. The factor α is used when the organism or tissue in the flask is surrounded by a liquid medium and was not necessary in this case where the organism, a grasshopper, was surrounded by air.

Volumenometer and its use.

The volume of the insects introduced into the flask, needed for calculation of the manometric constant, was determined by use of the volumenometer shown in Figure 1. In this instrument the volume of the object is measured by the volume of liquid it displaces when completely submerged.

Capillary tubing with an inside diameter of approxi-

Figure 1. Displacement Type Volumenometer.


Cork Stopper
1"
1½"
1¼"
A
½"
F
⅜"
B
1"
16"
Calibrated
Pipette
Rubber
Tubing
T Stopcock
D
2"
C
⅜"
E
Side View

mately 1 mm. was used for the stem and side-arms of the instrument.

The whole instrument was mounted on a board; it was supported and held away from the board slightly by blocks of appropriate length to which it was fastened by straps of rubber tubing.

Any suitable liquid may be used in the instrument. The author used acetone because this liquid seemed to wet the grasshoppers readily and completely without trapping air-bubbles on or around them. A calibrated pipette suitable to the volume to be measured was chosen and attached to the lower side-arm (D) of the instrument by means of rubber tubing. Acetone was poured into the bulb (A). If the volume of small insects was to be measured, only the lower part of the bulb (B) was filled. By opening the T stopcock (C) the liquid was allowed to flow around and rise in the calibrated pipette to the O mark; then the stopcock was closed. Acetone was then added to bring up the level in the bulb, and the level was carefully adjusted to one of the calibration marks of the side-arm (F) of the bulb. The insect to be measured was placed in the bulb, thus causing the level of the liquid to rise. The stopcock was then opened, allowing the liquid to rise in the calibrated pipette until the level of the liquid in the side-arm of the bulb returned to the mark to which it had been previously adjusted; the stopcock was then closed. The volume of the insect was then read in the

calibrated pipette. A cork stopper was used in the bulb to inhibit any evaporation of the acetone during the measurement.

The instrument offers the advantage of using a calibrated pipette suitable in accuracy to the volume to be measured. If the insect floated in the acetone, it was placed in a screen cage the volume of which had been previously determined. The author in his measurements used the same number of insects for a single measurement that were used in a manometric flask for that particular instar. This gave an average volume for that number of individuals of that instar.

B. Experimental Temperatures

Selection of experimental temperatures.

According to Parker and Shotwell (1932) and Shotwell (1941) the threshold temperature for spontaneous movement in Melanoplus differentialis occurs at the minimum of 68° Fahr. In order to study the relationship of rate of metabolism to the threshold temperature for spontaneous movement, the experimental temperatures were based on 68° Fahr. as a starting point.

Use of the Fahrenheit scale.

The Fahrenheit scale was chosen for experimental tempera-

tures, because the results can be more easily visualized in terms of field conditions than if the temperatures had to be translated from the Centigrade scale.

Temperatures used.

The highest temperature used was 80° Fahr. It lies well beyond the threshold temperature for spontaneous movement and is in the range for the usual field activities. The temperatures of 70° Fahr. and 65° Fahr. fall just on each side of the threshold temperature. The lowest temperature used was 40° Fahr. While this level is not the lowest temperature grasshoppers might be subjected to in the field, it is a fairly low field temperature for the season of the year during which grasshopper nymphs or adults are present, and development would certainly be at a low ebb at 40° Fahr. Temperatures of 50° Fahr. and 60° Fahr. were chosen as convenient intervals to complete the picture.

C. Experimental Technique

Three measuring respirometers were set up at a time, and one other respirometer was placed in the water bath to serve as a thermobarometer.

Use of several 'hoppers in each manometric flask.

As previously mentioned, several individuals, depending on the size of the instar and the volume of the manometric

flask, were used in a respirometer. This resulted in average readings, which were satisfactory for the purpose of the experiment, and it was felt that fluctuations between respirometers would be less than with single individuals. Also, the length of respiratory observation could be shortened, particularly with the higher temperatures where the grasshoppers would be inclined to become restless and move about in search of food if subjected to prolonged confinement in the respirometer.

Handling of the grasshoppers.

Selection of the grasshoppers from the cages was entirely at random. Grasshoppers were kept in separate cages after their use in the respirometers, but only those individuals which had not been previously used were taken for new observations. Great care was taken in handling the grasshoppers to avoid injuring them. First, second and third instars were picked up with an oral aspirator using as gentle suction as possible. Older instars and adults were very carefully picked up by hand.

Determination of weight of grasshoppers.

The grasshoppers were weighed to 1 mg. as a group on a balance accurate to 0.1 mg. During the weighing and the operations of placing the grasshoppers in the manometric flasks, all transfers were made by shaking the grasshoppers

from one receptacle into the other. A funnel was used in those cases where it was of help.

Movement of 'hoppers while in manometric flask.

No mechanical or confining devices were used to restrict the movements of the insects when they were in the manometric flask. It was felt that such precautions were not necessary or desirable. When grasshoppers are bound, confined or restricted in movement, they will struggle to escape; these struggling movements may produce a much greater source of error than would be found with the slight and occasional crawling movements of unrestricted 'hoppers.

While definite means were not taken to prevent movement on the part of the grasshoppers in the manometric flasks, excitatory stimuli such as light, sudden movements nearby, etc. were eliminated as much as possible. The respirometers were not shaken during the observation; there seemed to be no need for this arrangement, and shaking would only serve to excite the insects. The interior of the water bath was semi-dark, and lights in the room were so arranged that they did not shine directly into the water.

Operation of the respirometer.

Setting up respirometer and use of 30 minute conditioning period to allow equalization of pressure within the respirometer due to change in temperature. After the grasshoppers were

placed in the manometric flask and the KOH added in the central well, the flask was connected to the manometer. The respirometer was then suspended on the side of a water bath, operating at the desired temperature, so that the flask was immersed in the water. The stopcock was left open for 30 minutes. This period allowed equalization of pressure within the respirometer due to change in temperature from that of room temperature to that of the water bath. Also, this interval gave the grasshoppers an opportunity to become accustomed to their surroundings, recover from the irritation and excitation of being handled during weighing and setting up the respirometers, and to quiet down.

A 15 minute preliminary period. At the end of the 30 minutes the liquid in the manometer was adjusted to the 150 mm. mark, and the stopcock closed. During the following 15 minutes any carbon dioxide which might have been present in the air or produced by the grasshoppers during the preliminary 30 minute conditioning period was taken up by the KOH.

A 15 minute interval used for determination of oxygen uptake. At the end of the above mentioned 15 minute interval, the stopcock was left closed; the level of the liquid in the right hand arm of the manometer was adjusted to the 150 mm. mark, and the reading of the liquid taken in the left hand arm of the manometer. At the end of a 15 minute interval the level of the liquid in the right hand arm of

manometer was again adjusted to 150 mm. mark, and the reading of the left hand arm of the manometer was taken.

D. Expression of Results

After correction by the manometric constant for that manometer, the results were recorded as oxygen uptake in c.mm./mg./15 min.; this was used as an index to the rate of metabolism.

Example of calculations used in corrected reading.

To illustrate the method used in obtaining oxygen uptake in c.mm./mg./15 min., the calculations used to obtain the first figure, 0.029, of the 40 degree temperature column of Table 2 are presented. The actual readings of the thermobarometer and the respirometer, and the corrected reading of the respirometer are shown immediately below.

	Thermobarometer	Respirometer	Corrected Reading
First reading	264 mm.	274 mm.	
Second reading	262 mm.	269 mm.	
Difference	-2 mm.	-5 mm.	-3 mm.

Changes in the atmospheric pressure during the observational interval must be subtracted from the reading obtained in the respirometer during the period to give the correct reading for the decrease in oxygen content of the manometric flask. Since the thermobarometer gives a reading of the change in atmospheric pressure during the observational period, its

reading must be subtracted from the reading for the respirometer to obtain the corrected reading for the respirometer. Subtraction of a minus 2 from a minus 5 leaves minus 3, the corrected reading. To obtain the actual amount of oxygen decrease in the manometric flask in cubic millimeters, the corrected reading of the respirometer must be multiplied by the manometric constant for that particular respirometer; see Dixon (1934). The manometric constant at 40° Fahr. for the respirometer used in the above mentioned measurement is 0.558. The minus sign of the corrected respirometer reading can now be dropped, as the sign is no longer of any value. Multiplication of 3 times 0.558 gives the result of 1.674 cubic millimeters of oxygen decrease in the manometric flask. The weight of 'hoppers used in this measurement was 0.057 grams, or 57 milligrams. The cubic millimeters of oxygen used, 1.674, divided by the weight of grasshoppers used, 57 milligrams, gives the quotient, .0293, corrected to .029.

E. Experiments Conducted

The oxygen uptake was measured for each instar of Melanoplus differentialis at 40, 50, 60, 65, 70 and 80 degrees Fahr. A separation of males and females was made in the adults.

Effect of age on the oxygen uptake of adult M. differentialis.

The oxygen uptake of adult M. differentialis males and

females was measured at 80° Fahr. at weekly intervals from the beginning of adulthood until death. Fifth instar nymphs were collected in the field and placed in a large stock cage. This was watched carefully each day. As soon as a new adult appeared it was placed in a dated cage; all adult males or females appearing on the same day were caged together. Each dated cage contained a set of males and females with the date of appearance of each marked on the cage. The oxygen uptake was taken, starting with the day of their appearance, at weekly intervals until death.

Oxygen uptake of adults of several other Orthoptera.

Measurements were also made of the oxygen uptake at 80° Fahr. of adult male and female Periplaneta americana (Linn.) (the American roach); Brachystola magna (Scudder) (the large lubber grasshopper); Schistocerca lineata Scudder (a grasshopper); and of female Scudderia furcata Brunner (a katydid).

Effect of starvation at certain temperatures on the oxygen uptake of second instar Melanoplus differentialis.

An investigation was made into the effect of forty-eight hours of starvation at 60°, 65°, and 70° Fahr. on the oxygen uptake of second instar nymphs of M. differentialis. The 5 c.c. manometric flasks were used, and three grasshoppers were used for each measurement. The grasshoppers were selected at random from a large stock cage of second instar nymphs,

and new sets of individuals were used for every observation. Nymphs were taken from the stock cage where food was available at all times and placed in small vials which were then placed in constant temperature cabinets operating at 60°, 65°, and 70° Fahr. Sets of nymphs were removed and observations made on the oxygen uptake at the ends of three, six, twelve, twenty-four and forty-eight hour intervals. Results are given in c.mm. oxygen uptake/mg./15 min.

V. RESULTS

The oxygen uptake in c.mm./mg.15 min. for Melanoplus differentialis (Thomas) at 40°, 50°, 60°, 65°, 70° and 80° Fahr. is given in Table 2 for first instar nymphs, in Table 3 for second instar nymphs, Table 4 for third instar nymphs, Table 5 for fourth instar nymphs, Table 6 for fifth instar nymphs and in Table 7 for adults. Figure 2 is a graphic presentation of the means for each instar and temperature of these tables.

In Figure 3 the relationship of the oxygen uptake, in c.mm./mg./15 min., of each instar at a given temperature is shown.

The oxygen uptake at 80° Fahr., in c.mm./mg./15 min., of adult Melanoplus differentialis at successive weekly intervals from beginning of adulthood until death for seven pairs of females and three pairs of males is given in Table 8.

In Table 9 measurements of the oxygen uptake, in c.mm./mg./15 min., at 80° Fahr. of adult male and female Schistocerca lineata Scudder, Brachystola magna (Scudder), Periplaneta americana (Linn.) and female Scudderia furcata Brunner are given.

Results of a brief and preliminary investigation of the effect of starvation on the oxygen uptake at 60°, 65°, and

70° Fahr. of second instar nymphs of Melanoplus differentialis are given in Table 10.

Table 2. Oxygen Uptake in cmm./mg./15 min. of First Instar Nymphs of Melanoplus differentialis at Various Temperatures.

Replications	Temperatures in Degrees Fahr.					
	40	50	60	65	70	80
1	.029	.060	.091	.235	.190	.408
2	.019	.074	.076	.129	.222	.343
3	.027	.062	.081	.125	.218	.379
4	.036	.071	.093	.138	.226	.384
5	.019	.063	.105	.152	.233	.412
6	.025	.072	.122	.138	.226	.331
Mean	.026±.006	.067±.006	.095±.016	.153±.041	.219±.015	.376±.033

Table 3. Oxygen Uptake in cmm./mg./15 min. of Second Instar Nymphs of Melanoplus differentialis at Various Temperatures.

Replications	Temperatures in Degrees Fahr.					
	40	50	60	65	70	80
1	.032	.061	.095	.145	.219	.296
2	.028	.041	.096	.135	.193	.400
3	.043	.058	.100	.161	.197	.323
4	.025	.050	.107	.142	.165	.305
5	.029	.045	.128	.151	.196	.404
6	.028	.051	.097	.134	.210	.301
Mean	.031±.006	.051±.007	.104±.012	.145±.010	.197±.018	.338±.050

Table 4. Oxygen Uptake in cmm./mg./15 min. of Third Instar Nymphs of Melanoplus differentialis at Various Temperatures.

Replications	Temperatures in Degrees Fahr.					
	40	50	60	65	70	80
1	.028	.028	.102	.098	.160	.313
2	.021	.044	.095	.096	.162	.231
3	.024	.027	.095	.116	.158	.274
4	.020	.036	.078	.127	.154	.249
5	.029	.025	.094	.095	.164	.248
6	.020	.036	.089	.095	.159	.268
Mean	024±.004	033±.007	.092±.008	105±.013	160±.003	264±.028

Table 5. Oxygen Uptake in cmm./mg./15 min. of Fourth Instar Nymphs of Melanoplus differentialis at Various Temperatures.

Replications	Temperatures in Degrees Fahr.					
	40	50	60	65	70	80
1	.031	.045	.080	.117	.168	.308
2	.016	.031	.082	.108	.189	.254
3	.028	.045	.101	.100	.134	.294
4	.014	.023	.062	.120	.165	.288
5	.022	.051	.069	.126	.117	.256
6	.013	.058	.074	.117	.161	.246
Mean	021±.007	.042±.012	.078±.013	.115±.009	.156±.025	.274±.025

Table 6. Oxygen Uptake in cmm./mg./15 min. of Fifth Instar Nymphs of Melanoplus differentialis at Various Temperatures.

Replications	Temperatures in Degrees Fahr.					
	40	50	60	65	70	80
1	.015	.048	.092	.097	.109	.143
2	.038	.034	.053	.102	.115	.193
3	.017	.051	.081	.103	.119	.139
4	.032	.046	.066	.093	.175	.212
5	.017	.047	.059	.094	.103	.229
6	.017	.047	.061	.136	.122	.218
Mean	.023±.009	.046±.005	.069±.014	.104±.016	.124±.025	.189±.038

Table 7. Oxygen Uptake in cmm./mg./15 min. of Young Male and Female Adults of Melanoplus differentialis at Various Temperatures.

Replications	Temperatures in Degrees Fahr.					
Males	40	50	60	65	70	80
1	.026	.022	.074	.083	.078	.146
2	.019	.021	.049	.062	.061	.168
3	.017	.028	.065	.069	.134	.127
4	.017	.024	.055	.075	.057	.212
5	.017	.026	.061	.071	.056	.167
6	.028	.019	.049	.073	.114	.139
Mean	.021±.005	.023±.003	.059±.009	.072±.006	.083±.033	.160±.030
Females						
1	.015	.026	.067	.149	.116	.182
2	.039	.029	.073	.084	.080	.231
3	.026	.029	.058	.075	.165	.218
4	.019	.026	.085	.082	.115	.143
5	.023	.028	.088	.131	.227	.215
6	.026	.032	.059	.084	.075	.202
Mean	.025±.008	.028±.002	.072±.012	.101±.031	.130±.057	.199±.031

Figure 2. Effect of Temperature on the Oxygen Uptake of Each Instar of Melanoplus differentialis.

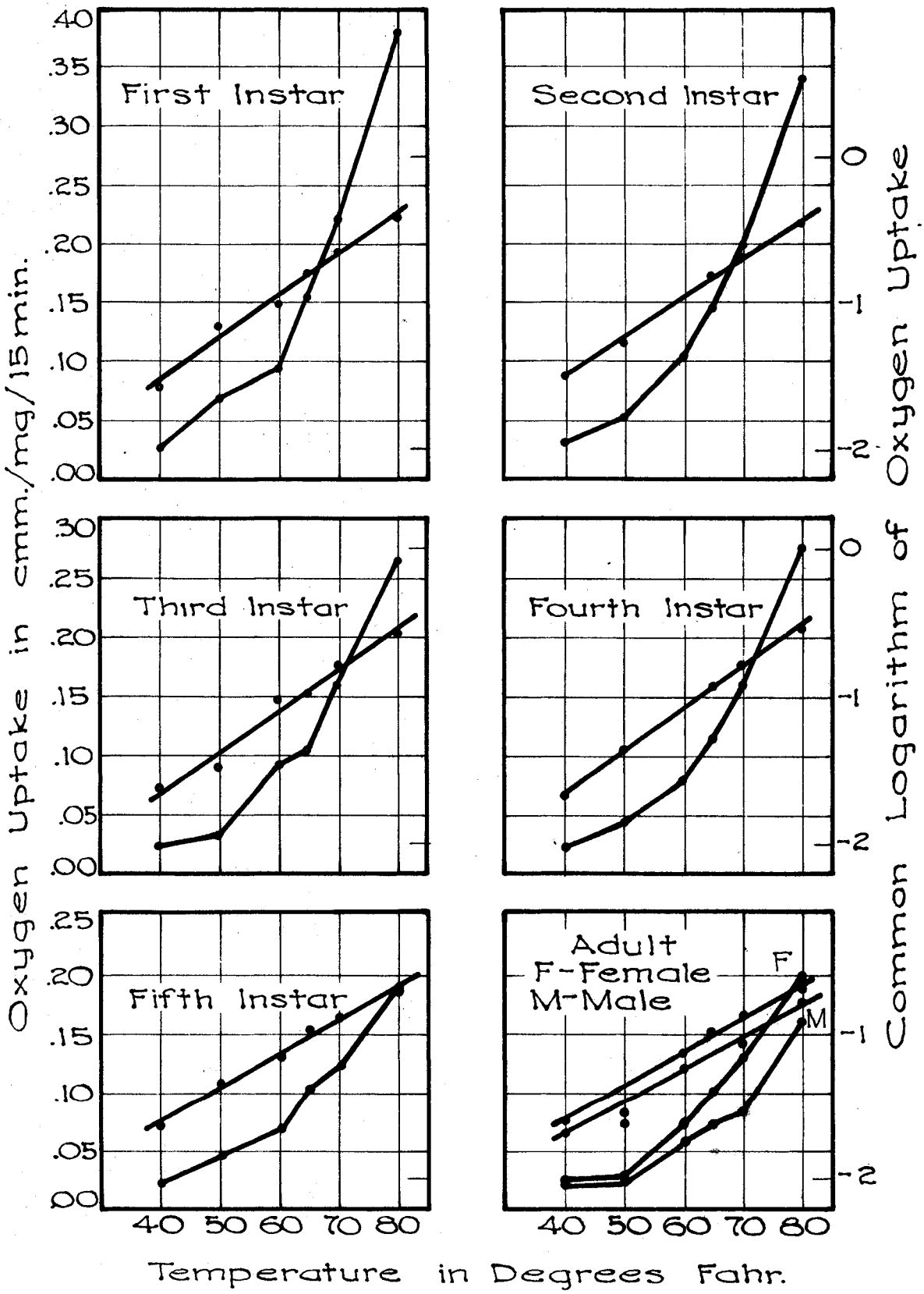

First Instar
Second Instar
Third Instar
Fourth Instar
Fifth Instar
Adult
F-Female
M-Male
F
M
.40
.35
.30
.25
.20
.15
.10
.05
.00
0
-1
-2
40 50 60 70 80
Oxygen Uptake in cmm./mg/15 min.
Common Logarithm of Oxygen Uptake
Temperature in Degrees Fahr.

Figure 3. Oxygen Uptake of the Various Instars of *Melanoplus differentialis* at Each Temperature.

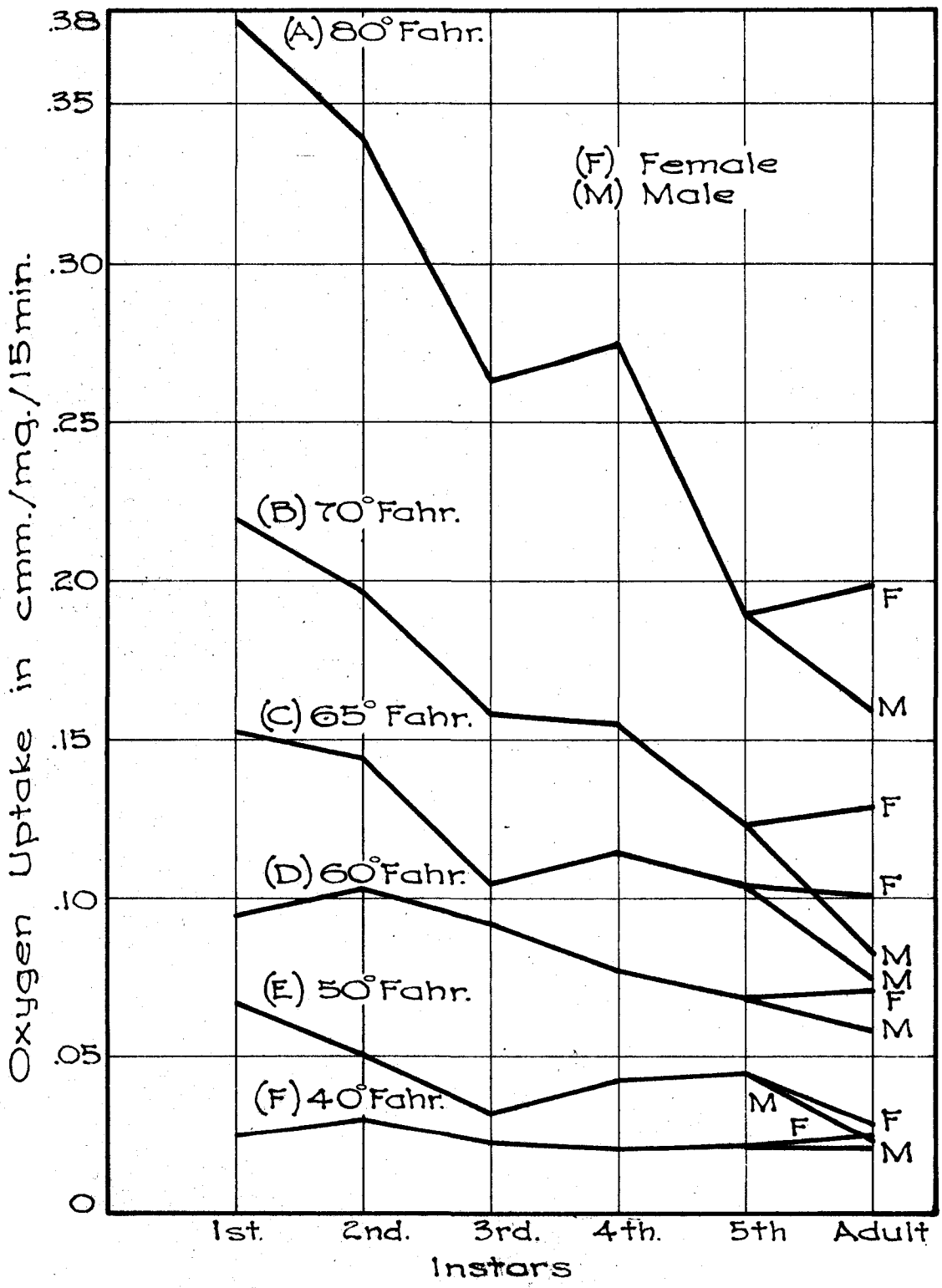

(A) 80° Fahr.
(F) Female
(M) Male
(B) 70° Fahr.
(C) 65° Fahr.
(D) 60° Fahr.
(E) 50° Fahr.
(F) 40° Fahr.
Oxygen Uptake in cmm./mg./15 min.
.38
.35
.30
.25
.20
.15
.10
.05
0
1st.
2nd.
3rd.
4th.
5th
Adult
Instars
F
M
F
F
M
M
F
M
M
F
F
M

Table 8. Effect of Age on the Oxygen Uptake in cmm./mg./15 min. of Adult *Melanoplus differentialis* at 80° Fahr.

Age in Weeks	Females								Males			
	A	B	C	D	E	F	G	Mean	A	B	C	Mean
Newly moulted adults	.208	.214	.169	.154	.209	.206	.191	.193±.023	.230	.174	.242	.215±.036
First week	.212	.188	.204	.144	.143	.231	.245	.195±.024	.172	.233	.119	.174±.056
Second week	.168	.184	.160	.175	.123	.191	.214	.174±.028	.236	.144	.184	.188±.046
Third week	.125	.127	.160	.158	.149	.203	.121	.150±.027	dead	.176	.153	.165±.016
Fourth week	.119	.142	.139	.205	.078	.182	.189	.151±.044		.159	.171	.165±.008
Fifth week	.168	.149	.137	.163	dead	.131	dead	.150±.015		.055	dead	.055
Sixth week	.171	.157	dead	dead		dead		.164±.009				
Seventh week	.098	dead						.098				
Eighth week	dead											

Table 9. Oxygen Uptake in cmm./mg./15 min. of Certain Male and Female Adult Orthoptera at 80° Fahr.

Insect		Male	Female
Schistocerca lineata		.151	.151
		.105	.086
(grasshopper)		.104	.112
		.098	.098
		.099	.111
		.159	.084
	Mean	.119 ± .027	.107 ± .024
Brachystola magna		.111	.046
		.078	.061
(grasshopper)		.067	.066
		.118	.054
		.112	.055
	Mean	.097 ± .023	.056 ± .007
Periplaneta americana		.126	.116
		.155	.212
(cockroach)		.204	.089
		.177	.131
		.163	.166
		.285	.157
	Mean	.185 ± .055	.145 ± .043
Scudderia furcata			.188
			.141
(katydid)			.189
			.171
			.229
			.196
	Mean		.186 ± .028

Table 10. Effect of Starvation, up to Forty-eight Hours, on the Oxygen Uptake in cmm./mg./15 min. of Second Instar Nymphs of Melanoplus differentialis at Temperatures of 60°, 65°, and 70° Fahr.

Temperature	Hours of Starvation					
	0	3	6	12	24	48
60 Fahr.		.128	.116	.069	.055	.089
		.054	.099	.111	.057	.057
		.085	.102	.096	.084	.128
Average	.1038	.089	.1056	.092	.0653	.0913
65 Fahr.		.081	.100	.143	.109	.181
		.123	.118	.150	.129	.217
		.093	.095	.141	.125	.355
Average	.1446	.099	.1043	.1446	.121	.251
70 Fahr.		.125	.153	.153	.069	.253
		.176	.187	.143	.066	.139
		.194	.171	.153	.057	.278
Average	.1966	.165	.1703	.1493	.064	.2233

VI. DISCUSSION

A. Effect of Temperature on the Oxygen Uptake of Melanoplus differentialis.

That an increase in temperature, at levels below the maximum, is accompanied by an increased rate of oxygen uptake by the grasshopper Melanoplus differentialis is shown by Figure 2, page 59. This relationship agrees with the results of Rogers (1929) and with the general conclusion that in insects there is an increased rate of metabolism with an increase in temperature until a certain limiting temperature is reached, whereupon further increases in temperature are accompanied by a decline in the rate of metabolism until death of the animal occurs.

Comparison of the oxygen uptake of the various instars of Melanoplus differentialis at 40° Fahr.

An analysis of variance, Snedecor (1938), (see Table 11, page 66), of the means of oxygen uptake at 40° Fahr. shows no significant difference between the various instars; F for 6 and 30 df is 1.37, and the 5 percent level of significance for these degrees of freedom is 2.42. Analysis of variance of the means of oxygen uptake at 50° Fahr. and also at 60° Fahr. (see Tables 12, page 66 and 13, page 67) shows

Table 11. Analysis of Variance Among the Means of Oxygen Uptake of the Various Instars of *Melanoplus differentialis* at 40° Fahr.

Source	df	Sum Squares	Mean Square
Total	41	2167.143	
Replications	5	98.295	19.657
Instars	6	446.809	74.468
Error	30	1622.049	54.068

F for Instars = 74.468/54.068 = 1.37 for 6 and 30 df.

Table 12. Analysis of Variance Among the Means of Oxygen Uptake of the Various Instars of *Melanoplus differentialis* at 50° Fahr.

Source	df	Sum Squares	Mean Square
Total	41	9850.286	
Replications	5	171.714	34.342
Instars	6	8029.952	1338.325
Error	30	1648.62	54.954

F for Instars = 1338.325/54.954 = 24.35 for 6 and 30 df.

Table 13. Analysis of Variance Among the Means of Oxygen Uptake of the Various Instars of Melanoplus differentialis at 60° Fahr.

Source	df	Sum Squares	Mean Square
Total	41	15260.405	
Replications	5	751.912	150.382
Instars	6	9433.905	1572.317
Error	30	5074.588	169.152

F for Instars = 1572.317/169.152 = 9.29 for 6 and 30 df.

a highly significant difference in the oxygen uptake of the various instars at both of these temperatures. At 50° Fahr. F for instars, at 6 and 30 df, is 24.35 and at 60° Fahr. F for instars, at 6 and 30 df, is 9.29. The 1 percent level of significance for 6 and 30 degrees of freedom is 3.47; so F for instars at 50° Fahr. and F for instars at 60° Fahr. are highly significant. The lack of significant difference in the means of oxygen uptake of the various instars of M. differentialis at 40° Fahr. is sharply contrasted with highly significant difference in the means of oxygen uptake of the various instars of M. differentialis at 50° Fahr.

The oxygen uptake of adult M. differentialis at 40° and 50° Fahr. In each instar with the exception of adults there is a greater oxygen uptake at 50° Fahr. than at 40° Fahr. Using Student's t-test, Snedecor (1938), to evaluate the difference in the means of oxygen uptake of adult male and of adult female M. differentialis at 40° and 50° Fahr.,

"t" equals 2.313 in the case of the males, and 1.489 for the females at 5 degrees of freedom. The "t" value at the 5 percent level of significance and 5 degrees of freedom is 2.571; so there is no significant difference in the means of oxygen uptake of adult M. differentialis at 40° and 50° Fahr. A comparison of the curves for third instar and adults in Figure 2, page 59, indicated a resemblance, but use of the t-test showed a significant difference in the means of oxygen uptake of third instar nymphs at 40° and 50° Fahr.

B. Relationship of Size to Oxygen Uptake

At 40° Fahr.

It is interesting to note (see Figure 3, page 61) that while the surface area of the body is a factor in the rate of metabolism of Melanoplus differentialis at 80° Fahr., it exerts little or no influence at 40° Fahr. All instars of M. differentialis are immobile at 40° Fahr., and it is apparent from the oxygen uptake that the rate of metabolism is at a low level. The fact that the great difference in body surface in relation to body weight between a first instar nymph and an adult female exerts no influence on the rate of metabolism suggests that the insect is in "chill-coma" with only enough activity in the body for the cells to remain alive.

No significant difference in the means of oxygen uptake

for adult M. differentialis at 40° and 50° Fahr. indicates that the adults are in "chill-coma" at 50° Fahr.

Application of Rubner's surface law to rate of metabolism of M. differentialis.

The curve of oxygen uptake for each instar at 80° Fahr. of Figure 3, page 61, is a good example of the application of Rubner's surface law to the rate of metabolism of insects, and the curves of Figure 3 show that the relationship is true at each of the other temperatures used in this investigation except 40° Fahr. where there is no significant difference in the means of oxygen uptake of the various instars.

The oxygen uptake of fourth instar nymphs of M. differentialis.

It seems worthwhile to point out that the oxygen uptake for fourth instar nymphs in the 80°, 70° and 65° Fahr. curves of Figure 3, page 61, is as high or higher than that of third instar nymphs. One would expect from the surface law relationship of Rubner that the oxygen uptake of fourth instar nymphs would be less than that of third instar nymphs as the fourth instar nymphs are larger and should have less body surface in relation to body weight. Other workers have met this same contradiction of expectations.

Bodenheimer (1929), working with Schistocerca gregaria, found a decrease in rate of metabolism between first and fifth instar nymphs, but third instar nymphs had a slightly

higher rate than expected. Also, there was an increase in rate of metabolism from fifth instar to young adults, and a decrease with age in adults. Results obtained by Butler et al. (1936) show that in Locusta migratoria there is a marked falling off in the rate of oxygen uptake per square centimeter of body surface from the first to the third instar, but that from fourth instar onwards to the adult stage the rate of oxygen uptake increased. Approximately as high an oxygen uptake was found for adults as for first instar nymphs, but the authors concluded that the surface law holds good for Locusta migratoria.

A consideration of the temperature curve for fourth instar Melanoplus differentialis in Figure 2, page 59, shows a regular curve in which the points fit the line with very good agreement. Since this is true and a parallel is shown in the 80°, 70°, and 65° Fahr. curves of Figure 3, page 61, a further investigation of this observation might be profitably made. While the present study offers no evidence upon which to base an explanation, it is conceivable that a turning point in the nymphal development of grasshoppers occurs with the third or fourth instar. One wonders if the greater oxygen uptake of fourth instar nymphs might be associated with sex differentiation and development of the reproductive organs. The writer suggests as a possible plan the measurement at 80° Fahr. of the oxygen uptake, carbon dioxide output, and determination of the R. Q. at bi-weekly intervals during the development of

a grasshopper from first instar to adult.

C. Rate of Metabolism at the Threshold Temperature for Spontaneous Movement of *Melanoplus differentialis*.

With the exception of the low oxygen uptake of adults at 50° Fahr., the oxygen uptake for each instar increases at a uniform rate with increase in temperature over the range of temperature used in this study. The observed points fit the line with good agreement, and the correlation coefficients for the lines are all greater than 0.9. Though the rate of metabolism, as shown by the oxygen uptake, is distinctly higher at 65° than at 60° Fahr., and at 70° than at 65° Fahr., there is no break or turning point indicated in the curve between 60° and 70° Fahr. So, while the rate of metabolism is higher at 68° Fahr. (the threshold temperature for spontaneous movement of *M. differentialis*) than it is at 67° Fahr. or at 65° Fahr., the rate of metabolism at the threshold temperature for spontaneous movement does not increase more than would be expected from the change in temperature, *i.e.*, a sudden increase or significantly higher rate of metabolism is not associated with the threshold temperature for spontaneous movement in *M. differentialis*. However, a certain rate of metabolism may be necessary before the stimulations which govern spontaneous movement in the grasshopper, *M. differentialis*, become effective. Also, the rate of metabolism in the cells of the nervous system might not parallel that of the body as

a whole. The relationship of the rate of metabolism of the cells of the nervous system to the threshold temperature for spontaneous movement might be masked in the measurement of the rate of metabolism of the body as a whole.

Relationship of the threshold temperature for spontaneous movement to starvation.

If food materials were unavailable to a group of insects, those insects having a higher rate of metabolism would use up the food reserves of their bodies faster and die from starvation in a shorter length of time. Individuals of M. differentialis, incapable of spontaneous movement and feeding at 60° Fahr., yet having a much higher rate of metabolism at 60° than at 50° Fahr., would surely starve to death in a shorter length of time at 60° Fahr. Since the rate of metabolism is still higher at 65° Fahr., the interval required for starvation would be even shorter. The difference in rate of metabolism with change in temperature is greater in lower instars than in older instars and adults, and the reserve food material per body weight less; so the effects of starvation would be more pronounced and death would come sooner in the early instars.

In grasshoppers present in an area favourable to their needs. In grasshoppers present in an area suited to their needs it is the younger instars, hatching and developing in the early spring, that are most likely to be subjected to temperatures lower than and bordering on the threshold temper-

ture for spontaneous movement, while older instars and adults living during the summer would find daily temperatures above this point and mostly favourable to their development and life activities. The nature of the weather in the spring is likely to be quite variable from year to year, and while in certain years the spring temperatures might be wholly favourable to hatching and development of grasshoppers, thus allowing populations to build up, such a year might be followed by one with an early warm spell followed by periods of coolness in which the starvation of grasshoppers at temperatures bordering on the threshold temperature for spontaneous movement might be a factor in reducing or wiping out the infestation.

The disappearance of infestations in local areas. The disappearance or reduction of grasshopper infestation in local areas has been noticed occasionally, as, for example, the occurrence mentioned on page 1. It is entirely possible that in some cases the temperature in that small area dipped below the threshold temperature for spontaneous movement long enough for the insects to be starved out while the temperature in the larger surrounding area remained above that point or rose above it for at least a part of the time.

In insects trying to establish themselves on the fringe of their environment or arriving in new areas. To what extent similar conditions of temperature and starvation might apply to insects trying to establish themselves on the fringe of their environment or arriving in new areas either voluntarily,

or through natural means such as wind or water, or through some man made mode of transportation remains unknown. Some such cases probably occur and pass unnoticed.

D. Difference in the Oxygen Uptake Between Sexes of Certain Orthoptera.

A comparison of the rate of metabolism of males and females by previous workers has shown that in some species the males have a higher rate and in others the females have the higher rate. The data of Table 9, page 63, indicate that for the species of Orthoptera investigated here the males have a higher rate of metabolism than the females.

The effect of temperature on the oxygen uptake of adult M. differentialis was studied during the first two weeks of their adult life. These data, presented in Table 7, page 57, show that young adult female M. differentialis have a higher rate of metabolism than the corresponding males. An inspection of Table 8, page 62, shows that after the second week of adult life the male M. differentialis have a higher rate of metabolism than adult female M. differentialis of the same age. The higher oxygen uptake for young adult female M. differentialis than for fifth instar nymphs, shown in Figure 3, page 61, and a decrease in oxygen uptake as the females become older, see Table 8, page 62, agree with the work of Bodenheimer (1929) on Schistocerca gregaria.

E. Oxygen Uptake During the Adult Lifetime of Female *Melanoplus differentialis*.

An analysis of variance of the means of oxygen uptake of female *M. differentialis* at weekly intervals shows no significant difference between the groups of females, but does show a significant difference in the means of oxygen uptake at weekly intervals. Perhaps the higher oxygen uptake of females during the first two weeks of adult life may be associated with the development of the ovaries and the formation of eggs. The lower oxygen uptake at the end of the third week in Table 8, page 62, is coincident with the time the females became gravid and began egg laying activities. The writer is of the opinion that the results presented in Table 8 only indicate a trend and that a further study of this problem is indicated. Measurement of oxygen uptake and carbon dioxide output and determinations of R. Q. at intervals during the life span of adult grasshoppers, correlated with observations on the time of copulation and egg laying, would furnish additional information on the rate of metabolism and the types of food metabolized during these various phases of adult life. Comparison of results from both sexes might provide an interesting point of information.

F. Effect of Forty-eight Hours of Starvation on Oxygen Uptake at Certain Temperatures of Second Instar Nymphs of *Melanoplus differentialis*.

An investigation of the effect of starvation at various temperatures on the rate of metabolism of second instar nymphs of *M. differentialis* was started. The results obtained are presented in Table 10, page 64. It is difficult to draw conclusions from these data, and this complicates any discussion. There seems to be an opportunity for interesting information. The writer feels that a further study of this matter should include measurement of both oxygen uptake and carbon dioxide output and determination of the R.Q. at the various intervals. The measurements should be made at regular intervals until the individuals die from starvation. It is of interest to note in Table 10 that while a decrease in oxygen uptake after twenty-four hours of starvation is indicated at 60° and 70° Fahr., which agrees with the expected decrease in rate of metabolism as starvation progresses, this trend is not shown in individuals starved at 65° Fahr.

VII. SUMMARY AND CONCLUSIONS

A review of the literature of respiratory metabolism of insects is presented and accompanied by a tabulated summary of measurements of the respiratory metabolism of insects for the period 1914 through 1941.

The writer used the grasshopper, Melanoplus differentialis, as the experimental insect. Eggs. of M. differentialis collected in the field were identified in the laboratory according to the method of Tuck (1939). After the diapause was broken, the eggs were placed in damp sand and incubated in a constant temperature cabinet operating at 30° Cent. The nymphs and adults were reared in Riley cages placed in a heated, sunlit greenhouse; they fed on potted plants of barley, wheat, corn and alfalfa placed in the cages.

The instars were separated according to the key for Melanoplus bivittatus devised by Shotwell (1941), and each instar was kept separate from the others.

The instrument used to measure the oxygen uptake was the Warburg manometer, a constant volume type of respirometer. Three sizes of Warburg flasks, 5 c.c., 15 c.c. and 150 c.c., with central well were used. First and second instar nymphs were placed in 5 c.c. flasks. The 15 c.c. flasks were used for third and fourth instar nymphs, and 150 c.c. flasks were

used for fifth instar nymphs and adults. The Warburg manometers were used according to directions given in Dixon (1934).

The Warburg manometers were suspended on the side of a water bath so that the flasks were immersed in the water during the intervals used to measure the oxygen uptake. Temperature control of the water bath was accomplished through the use of a quick-set bi-metallic thermostat. Three blade type heating elements provided the heating unit. When temperatures below that of room temperature were desired a cooling unit consisting of a pump to circulate water from a cold coil, surrounded by cracked ice, outside the bath to a coil in the bath through the pump and back to the cold coil was used.

The volume of the insects introduced into the flask, needed for calculation of the manometric constant, was determined by the use of a volumenometer. A figure showing details of the volumenometer is provided and directions for its use are given.

According to Parker and Shotwell (1932) and Shotwell (1941) the threshold temperature for spontaneous movement in Melanoplus differentialis occurs at 68° Fahr. In order to study the relationship of rate of metabolism to the threshold temperature for spontaneous movement, the oxygen uptake was measured for each nymphal instar and for young adults of M. differentialis at the controlled temperatures of 40°, 50°, 60°, 65°, 70° and 80° Fahr.

The number of grasshoppers which could be accommodated without crowding in a manometric flask suitable to their size was determined to be ten first instar or five second instar nymphs in a 5 c.c. flask, five third instar or three fourth instar nymphs in a 15 c.c. flask and five fifth instar or two adults in a 150 c.c. flask. The use of several individuals in a respirometer flask resulted in average readings, but enabled the author to use a short interval for the measurement of oxygen uptake. Also, it was felt that fluctuations between respirometers would not be as great as if single individuals were used.

Groups of grasshoppers to be used in the measurement of oxygen uptake were selected at random from a stock cage. They were weighed to 1 mg. on a balance accurate to 0.1 mg.

Care was taken to avoid injuring the grasshoppers while handling them in selection, weighing and placing them in the respirometers.

After the grasshoppers were placed in a respirometer properly arranged to measure oxygen uptake, the respirometer was placed on the water bath. The stopcock was left open for 30 minutes to allow equalization of pressure within the respirometer due to change in temperature from that of room temperature to that of the water bath. This interval also gave the grasshoppers an opportunity to become accustomed to their surroundings, recover from the excitation of being handled, and quiet down.

At the end of the 30 minutes the liquid in the manometer was adjusted to the 150 mm. mark, and the stopcock closed. During the following 15 minutes any carbon dioxide which might have been present in the air or produced by the grasshoppers during the preliminary 30 minute conditioning period was taken up. At the end of this 15 minute interval the stopcock was left closed; the level of the liquid in the right hand arm of the manometer was adjusted to the 150 mm. mark, and the reading of the liquid taken in the left hand arm of the manometer. Observation of the respiratory metabolism was taken for the following 15 minute interval.

After correction by the manometric constant, the results were recorded as oxygen uptake in c.mm./mg./15 min.

In addition to a study of the relationship of the rate of metabolism to the threshold temperature for spontaneous movement, the oxygen uptake of adult Melanoplus differentialis males and females was measured at 80° Fahr. at weekly intervals from the beginning of adulthood until death. Also, measurements were made of the oxygen uptake at 80° Fahr. of adult male and female Periplaneta americana (Linn.), Brachystola magna (Scudd.), and Schistocerca lineata Scudder, and of female Scudderia furcata Brunner.

The results are presented in tables. Graphs showing the oxygen uptake of each inster at various temperatures and the oxygen uptake of the various instars at each temperature are given. The following conclusions may be drawn:

1. The oxygen uptake of the five nymphal instars of Melanoplus differentialis increases at a uniform rate with increase in temperature between 40° and 80° Fahr.

2. No significant difference is shown in the means of oxygen uptake of adult Melanoplus differentialis at 40° and 50° Fahr.

3. The rate of oxygen uptake indicates that a sudden increase or significantly higher rate of metabolism is not associated with the threshold temperature for spontaneous movement in Melanoplus differentialis; or, that as the temperature approaches the threshold temperature for spontaneous movement the rate of metabolism does not increase more than would be expected from the change in temperature.

4. The rate of oxygen uptake at 80° Fahr. during the growth of Melanoplus differentialis from first instar to adult is in agreement with Rubner's Surface Law.

5. Contrary to the expectations in accordance with Rubner's Surface Law the means of oxygen uptake of instars of Melanoplus differentialis at 40° Fahr. do not differ significantly.

6. The oxygen uptake of fourth instar nymphs of Melanoplus differentialis at each of 65°, 70°, and 80° Fahr. is higher than would be expected from the surface law relationship.

7. During the first two weeks of adult life, female Melanoplus differentialis have a higher oxygen uptake than

males of the same age; after adult Melanoplus differentialis are three weeks old the oxygen uptake of males is higher than that of females.

8. The males of Schistocerca lineata, Brachystola magna and Periplaneta americana have a slightly higher rate of metabolism at 80° Fahr. than do the females.

9. A significant decrease at the third week is shown in the means of oxygen uptake of female Melanoplus differentialis measured at successive weekly intervals during adult life. It is suggested that this is perhaps due to the high rate of oxygen uptake at the beginning of adult life in the development of the ovaries and formation of the eggs followed by the gravid condition and beginning of egg laying activities at about the third week of adult life.

VIII. LITERATURE CITED

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