Pakistan J. Zool., vol. 41(2), pp. 143-148, 2009.

A Comparative Study on the Morphology of Egg Pods, Egg Development and Hatching of Three *Hieroglyphus* Species (Acrididae: Orthoperta)

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Abstract. - The egg-pods of three grasshopper species *i.e., Hieroglyphus perpolita* (Uvarov), *H. oryzivorus* Carl and *H. nigrorepletus* I. Bolivar were studied under laboratory conditions during 2005-2006 from Pakistan. Significant differences were found in the sizes of the pods and number, distribution pattern and weight of eggs in the pods. The pods are sub-cylindrical and slightly curved. The weight (3.64±0.60g) and width (22.82±2.21 mm) of egg pods of *H. perpolita* is significantly greater than that of pods of *H. oryzivorus* (1.30±0.03 g) and *H. nigrorepletus* (0.185±0.003 g) whereas pods length of *H. oryzivorus* (34.68±0.84 mm) is significantly greater than that of *H. perpolita* (27.78±0.78 mm) and *H. nigrorepletus* (18.38±1.15 mm). Eggs cylindrically elongated, large, slightly bent in the middle and rounded at ends. The length (6.37±0.06 mm) and weight (0.128±0.01 mm) of eggs of *H. perpolita* is significantly greater than that of *H. oryzivorus* (5.4±0.1 mg and 4.52±0.07 mm, respectively) and *H. nigrorepletus* (5±0.06 mg and 4.55±0.0063 mm, respectively). Likewise there was significant difference between the weight of dry eggs and fresh laid eggs of *H. perpolita.* The egg-pod is full of eggs without any empty space. There are more eggs at the base and their number decreased towards the top. The major hatching occurr within few weeks *i.e*., 57.43 to 100% in *H. perpolita* from June to August but mostly in the month of June, 76.85% to 100% in *H. oryzivorus* from July to September but mostly in August, and 69.09% in *H. nigrorepletus* from July to August, mostly in mid July then hatching stopped.

Key Words: *Hieroglyphus,* morphology, egg-pods, development, hatching.

INTRODUCTION

Three grasshopper species *Hieroglyphus perpolita* (Uvarov), *H.oryzivorus* Carl, and *H. nigrorepletus* I. Bolivar are the most important pests of cultivated crops of primarily Asian distribution *e.g.*, Pakistan (Janjua, 1957; Riffat *et al.,* 2007; Riffat and Wagan, 2008), India (Uvarov, 1922; Roonwal, 1976), Thailand, Bangladesh, China, Afghanistan (Mason, 1973). Like many other grasshopper species, *Hieroglyphus* are also univoltine and over-winter as eggs in soil. Generally, their eggs are deposited from late July to mid November, but the hatching time of first instars nymphs are species specific from mid June to early August (Wagan and Riffat, 2006).

Several authors have described egg-pods of acridoids and discussed their structure in relation to their ecology in the field (Zimin, 1938; Waloff, 1950; Khalifa, 1956; Chapman and Robertson, 1958; Katiyar, 1960; Descamps and Wintrebert, 1966). Certain other aspects, including oviposition and mating, food selection, life-history, identification and pest status of *Hieroglyphus* species has been studied by Srivastava (1956), Pradhan and Peswani (1961), Siddiqui (1986, 1989), Wagan and Riffat (2006) and Riffat and Wagan (2007a,b,c,d, 2008). However, morphological variation in egg-development and hatching of these three *Hieroglyphus* species has not received much attention from Pakistan. An attempt has therefore, been made to study the morphological difference in egg-pods, egg development and factors influencing hatching of *Hieroglyphus* species.

The information given by Janjua (1957) from Pakistan on the egg-pod of *H. oryzivorus* and that of Roonwal (1976) on egg-pod of *H. nigrorepletus* has not been very adequate. there was no such information available regarding *H. perpolita* which prompted the present study.

MATERIALS AND METHODS

Collection and rearing of samples

The stock of *Hieroglyphus* adults were collected from the agriculture fields of rice, maize,

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0030-9923/2009/0002-0143 $ 8.00/0

sugarcane, millets, fodder crops and their surrounding vegetation of grasses with the help of traditional insect hand-net (8.89 cm in diameter and 50.8 cm in length) as well as by hand picking. The collection was made during the year 2005-2006 in the months of June to November from various provinces of Pakistan. Collected material was brought to the laboratory and caged in rearing box (length 16½, width 13½ cms) as well as in separate ordinary jam bottles, in pairs. Then these cages were placed under laboratory (25°-23/N 68°-24/ E) conditions where the temperature fluctuated between 28±2°C to 39±2°C with relative humidity of 26-61%. These temperature and relative humidity regimes were similar to the field conditions.

Collection of egg-pods and eggs

Each cage was provided with cup containing sieved garden sand for oviposition. Fresh drops of water were added daily to keep the sand moist. Green shoots of fresh maize leaves were clipped and placed in 50ml conical flask filled with water. Experimental cages and jars were thoroughly cleaned and placed in the sunlight for two to three hours after 10-12 days. The paper sheet placed on the bottom of the cage was changed daily. All egg cups were checked daily in the morning. Egg-pods were collected and opened carefully by following the method of Pradhan and Peswani (1961). The number of eggs, size and arrangement in each egg-pod was recorded. Same method was adopted for all the species studied *viz*., *H. perpolita, H. oryzivorus* and *H. nigrorepletus.*

Hatching and development of eggs

Fifty egg-pods of each species were immersed in the soil. Soil mixture was geared up by addition of two parts of ordinary field soil, one part of sand and a little of fine road gravels. The soil combination was put in earthen pots and small brick pieces were kept over the hole at the bottom of pots for proper drainage of excess water, the egg-pods were subsequently buried at a depth of about one inch and in each pot 10 pods were placed under laboratory condition. The water was sprinkled with the watering cans without disturbing the position of the egg-pods or soil level in the pots. From these specially prepared pots the hoppers could emerge out successfully. All the experiments described below concerning hatching of eggs and emergence of hoppers was carried out in these specially prepared pots in the laboratory.

Statistical analysis

Data obtained from experimental groups were subjected to one-way analysis of variance (ANOVA) (SPSS 10.0 Soft-Ware) with repeated measures and significant means were determined using Duncan's New Multiple Range Test (DNMRT) and treatment means were compared using the Least Significant Difference Test (LSD).

RESULTS AND DISCUSSION

Morphology of egg-pods and eggs

Female usually lays a heap of eggs in grounds, eggs usually enclosed in a sac, consisting of the hardened secretion cemented with grains of earth. The pods are sub-cylindrical and slightly curved. The lower end is rounded and convex and the top slightly concave; the latter part is more fragile partly composed of dried frothy material, the plug, and has a tendency to break off. Egg-pod-size and variation of the eggs have been shown in Tables I and II for *H. perpolita, H. oryzivorus* and *H. nigrorepletus*. The weight and width of *H. perpolita* is significantly greater than that of *H. oryzivorus* and *H. nigrorepletus*, whereas length of *H. oryzivorus* (Fig. 1G) is significantly greater than that of *H. perpolita* and *H. nigrorepletus* (Figs. 1A,D).

In *H. perpolita* eggs in the egg-pod are arranged radially so that their micropylar ends are visible all around the pod (Fig. 1B), whereas in *H. nigrorepletus* and *H. oryzivorus* eggs are arranged bilaterally symmetrical *i.e*., the micropylar ends are visible at only one end (Fig. 1E, F, H, I). However, in some cases there was no proper arrangement of eggs in the egg-pod.

Eggs are cylindrically elongated, large slightly bent in the middle and rounded at ends. The chorion covering the eggs is thin and almost colorless and has a weak hexagonal sculpturing except at the posterior end (lower end in the natural position) which is dark, brown and has a thick-walled hexagonal sculpturing. Just above this band lies a ring funicular canals, the micropylar canals, as

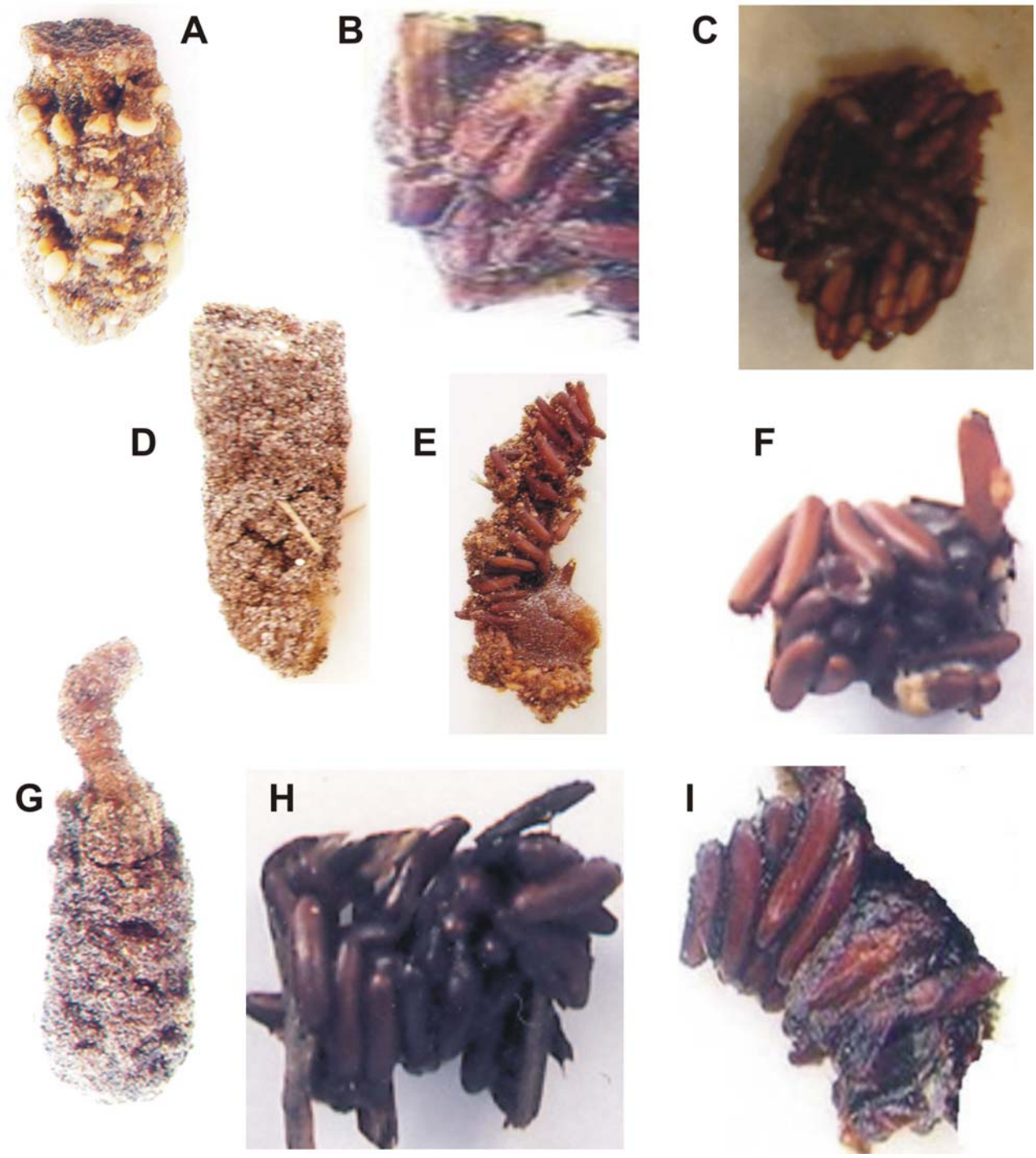

Fig. 1. A-C: *Hieroglyphus perpolita* (Uvarov); A, enlarged view of an egg pod; B, side view of egg pod; C, section across the pod; showing arrangement of eggs in the pod.
D-F: *Hieroglyphus nigrorepletus* ,I.Bolivar; D, enlarged view of egg pod; E, lateral view and F, ventral view of egg pod showing arrangement of eggs in the pod.
G-I: *Hieroglyphus oryzivorus,* Carl; G, enlarge view of the pod; H, ventral view and I, lateral view of egg pod showing arrangement of eggs in the pod.

Table I.- Measurement of egg-pods of *Hieroglyphus* spp.

Species	Weight (g) (Mean±SD)	Length (mm) (Mean±SD)	Width (mm) (Mean±SD
H. perpolita	3.64±0.60 [c]	27.78±0.78 [b]	22.82±2.21 [c]
H. oryzivorus	1.30±0.03 [b]	34.68±0.84 [c]	10.13±0.24 [a]
H. nigrorepletus	0.185±0.003 [a]	18.38±1.15 [a]	12.16±1.55 [b]
$F_{.(0.05)}$	$_{(1.70)}$ 04.36*	$_{(26.94)}$ 48.00 *	$_{(15.03)}$ 27.05 *

Mean (±SD) in the same column followed by the same letters do not differ significantly p≤ 0.05 (DNMRT)

Table II.- Measurement of eggs of *Hieroglyphus* spp.

Species	Freshly laid eggs (n=15)			Dry eggs (n=15)		
	Weight (Mean± SD) (mg)	Length (Mean± SD) (mm)	Width (Mean± SD) (mm)	Weight (Mean± SD) (mg)	Length (Mean±SD) (mm)	Width (Mean±SD) (mm)
H. perpolita	0.128±0.01 [b]	6.37±0.06 [a]	2.17±0.12 [a]	0.10±0.01 [a]	6.35±0.29 [a]	1.7±0.02 [a]
H. oryzivorus	0.0054±0.0001 [a]	4.52±0.07 [a]	1.45±0.0063 [a]	0.005±0.00003 [b]	4.46±0.50 [a]	1.45±0.006 [a]
H. nigrorepletus	0.005±0.00006 [a]	4.55±0.0063 [a]	1.45±0.008 [a]	0.0065±0.00007 [b]	4.53±0.004 [a]	1.45±0.007 [a]
$F_{.(0.05)}$	$_{(0.046)}$ 00.87*	$_{(5.14)}$ 09.60 [ns]	$_{(1.69)}$ 04.36 [ns]	$_{(0.03)}$ 00.87*	$_{(5.11)}$ 09.60 [ns]	$_{(1.53)}$ 04.36[ns]

Mean (±SD) in the same column followed by the same letters do not differ significantly p≤ 0.05 (DNMRT).

Table III.- Summary of egg hatching of *Hieroglyphus* spp. in Ist year after egg-laying.

Species	Approximate period from egg-laying to hatching (Mean ±SD) (Days)		No. of hopper hatch and % of hatching) In the lot		% of hatched	Average per pod
	Minimum	Maximum	Average	Range		
H. perpolita	205.05±50.05	299.46±38.41	130.11	83-227	57.43-100%	21.21
H. oryzivorous	247.79±45.96	300.32±31.77	210.37	137-319	76.85-100%	33.22
H. nigrorepletus	267.19±23.25	299.20±26.30	119.87	93-169	69.09-100%	23.21

the embryo developed, it secretes on the inside a thick, elastic cuticle which protects the embryo until hatching.

Table II shows the size, length and weight of eggs of *H. perpolita* is significantly greater than that of *H. oryzivorus* and *H. nigrorepletus*. Similarly there was significant difference between the weight of dry eggs and fresh laid eggs of *H. perpolita.* The egg-pod is full of eggs without any empty space. There are more eggs at the base and their number decreased towards the top.

Chapman and Robertson (1958) reported two types of egg arrangements in the egg-pod (i) the eggs radially arranged so that their micropyler ends are visible all-around the pod (ii) the eggs bilaterally symmetrical arranged so that their micropyler ends are visible on only one side of the pod. Uvarov (1977), however, reported some variation of these arrangements. Furthermore the arrangement of the eggs is often lost when in the fragile pod is dugout. Our findings, however, generally agree with Chapman and Robertson (1958), Katiyar (1960), Roonwal (1976) and Uvarov (1977). The structure of egg-pod described for *H. nigrorepletus* agreed with Roonwal (1976) and not with that of Pruthi (1949), Narayanan and Samuel (1954) and Pradhan and Peswani (1961). The shape and arrangement of eggs in pod for *H. perpolita* and *H. oryzivorus* is being reported for the first time. Uvarov (1966) reported that the size of newly laid egg mostly depends on the size of species; its length in small species is greater in relation to the female body length than in large. In the present study variation reported in the egg size of *H. perpolita* also agrees with that of Uvarov (1966). Present study suggests that significant difference in the egg-pods of

Hieroglyphus might be due to the oviposition habitat of species particularly the soil conditions.

Egg-pods size of *H. nigrorepletus* has been variously given as follows: 16.8mm X 6.6mm (n=30) (Chaturvedi, 1946), 17.8mmX 9.4mm, (Pradhan and Peswani, 1961). The variation is also reflected in weight. The sun-dried pods weigh 0.58-1.37 g which nearly agrees with that of Pradhan and Peswani (1961). Our present length (4.55±0.0063 mm) and width (1.45±0.008 mm) of freshly laid egg, and length (4.53±0.004 mm) and width (1.45±0.007 mm) of dry egg agrees with the dimensions given by Roonwal (1976).

Egg development and hatching

For the development of eggs, fifty egg-pods of *H. perpolita, H. oryzivorus* and *H. nigrorepletus* were kept in separate soil in earthen pots during June to onward. Major hatching occurred within few weeks *viz*., 57.43 to 100% in *H. perpolita* from June to August but mostly in the month of June, 76.85% to 100% in *H. oryzivorus* from July to September, mostly in August; and 69.09% to 100% in *H. nigrorepletus* from July to August, mostly in mid-July. Then hatching stopped. Total developmental period recorded was 205.05±50.05 to 299.46±38 days for *H. perpolita,* 247.79±45.96 to 300.32±31.77 days for *H. oryzivorus* and 267.19±23.25 to 299.20±26.30 days for *H. nigrorepletus* (Table III). The present study shows that *H. perpolita* hatch earlier than the other two species and the hatching percentage was significantly higher during the month of July, August and September as compared with other species of *Hieroglyphus*. The first instar hatches out as vermiform larva enveloped in a fine membrane. It makes a very fine bore of pin head diameter in the soil making worm like movements with the help of abdomen. After reaching the surface it casts. These skins cast can be counted at the mouth of the hole through which the hoppers made their way out.

The results of present study confirms the previous findings of Roonwal (1945, 1976), Pruthi (1949, 1969), Anonymous (1950) and Pradhan and Peswani (1961) who have observed 10-11 months long egg diapause in the embryonic stages of *H. nigrorepletus* as against no diapause reported by Saxena (1948) and Narayanan and Samuel (1954). A diapause phenomenon in *H. perpolita* and *H. oryzivorus* is being reported for the first time. The majority of eggs show one year diapause, while only 10-11% eggs go to prolonged diapause *i.e.*, 20-23 months after egg-laying even though watered regularly. Similar results were also reported by Roonwal (1976) for *H. nigrorepletus.*

Hieroglyphus species in Pakistani grassland hatch in different periods of summer (Wagan and Riffat, 2006). The difference between the emergences and duration of hoppers might be because of insect's habitat, seasonal fluctuation and egg diapause which vary from place to place depending on climatic and ecological conditions of the region. Furthermore, present study also revealed that rains in June and July are important, because if these two months were dry, a large percentage of the eggs would fail to hatch. On the whole, early and uniformly distributed summer rains create favorable conditions for this species.

Pradhan and Peswani (1961) observed that hatching of the eggs of *H.nigrorepletus* is governed by three main factors namely, moisture, soil and season. If any of these factors was missing, the eggs did not hatch. The present study agrees with this observation.

ACKNOWLEDGEMENTS

Funding for the research, provided by Pakistan Science Foundation Islamabad under research project No. PSF S-SU/ Bio 338 is gratefully acknowledged.

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(Received 22 July 2008, revised 17 December 2008)