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A physiological age-grading system for female *Hydrellia pakistanae* Deonier (Diptera: Ephydridae)

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Abstract

The female reproductive system of *Hydrellia pakistanae* Deonier was described by dissecting individuals of known ages and describing changes in relation to number of eggs oviposited. Female *H. pakistanae* have meroistic ovaries, in that specialized nurse cells or trophocytes (nutritive cells associated with the eggs) are present within each ovariole, and of the polytrophic subtype because nurse cells closely accompany each developing egg, or oocyte. The reproductive system includes two ovaries, each consisting of 8 or 10 tube-like ovarioles. The ovariole can be divided into two distinct areas: a distal germarium, which produces the follicles, and a more proximal vitellarium, which houses the developing follicles. Each ovariole is surrounded by an ovariole sheath and houses several developing follicles, a term referring to the ova (yolk and surrounding cells; i.e., the egg), nurse cells, and surrounding epithelium. Within an ovariole, the follicle proximate to the lateral oviduct is the most mature with each subsequent, more distal follicle becoming less mature. All ovarioles within an ovary are connected via the lateral oviducts, while the common oviduct connects the ovaries to each other. The spermatheca and accessory glands branch off the common oviduct. Sperm stored in the spermatheca fertilize eggs as they pass through the common oviduct. Based on this information the continuum of ovarian maturation was divided into three nulliparous and four parous stages. The nulliparous stages are classified based on degree of ovariole segmentation and maturity of the most proximal follicle while follicle relic quantity and appearance is mainly used to separate the parous classes. Limited field sampling demonstrated the possibility of using this system to assess reproductive health of wild populations.

Keywords: *Hydrellia pakistanae*; *Hydrilla verticillata*; Biological control; Age-grading; Follicular relics

1. Introduction

Hydrellia pakistanae Deonier, an introduced leaf-mining fly species in the family Ephydridae, is a potentially valuable organism for the biological control of *Hydrilla verticillata* (L.f.) Royle, an invasive aquatic plant. Native to Pakistan, India, and as far north as Beijing, China, *H. pakistanae* was first released in southern Florida in 1987. Currently, it has been released at over 50 sites in Alabama, Arkansas, California, Florida, Georgia, Louisiana, North Carolina, Virginia, and Texas (Center et al., 1997). *H. pakistanae* is now established in Arkansas, Louisiana, Florida, Georgia, and Texas (Center et al., 1997) with range expansions noted in both Texas and Louisiana (Grodowitz et al., 2000; Julie Nachtrieb, US Army Engineer Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX, personal communication and Jan Freedman, US Army Engineer Research and Development Center, Vicksburg, MS, personal communication. The larvae of *H. pakistanae* feed exclusively on hydrilla, and while they have been shown to impact photosynthesis and cause significant damage to experimental populations (Doyle et al., 2002, 2007), there are conflicting opinions as to their impact on field populations of hydrilla. *H. pakistanae* appears to be successful at

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controlling hydrilla in Texas field populations (Grodowitz et al., 1999); however, fly populations at many Florida sites have only achieved a maximum of 15% leaf damage to hydrilla, with many sites exhibiting much lower population levels (<200 immatures/kg) and associated damage (Wheeler and Center, 2001). Recent work by Grodowitz et al. (2004) has characterized the impact of the fly to both experimental and field populations.

Reasons for such low population levels at many sites are unknown but several ideas have been cited. These include parasitism by a native pupal parasite (*Trichopria columbiana* Ashmead (Hymenoptera: Ichneumonidae) (Wheeler and Center, 2001; Doyle et al., 2002; Christie Snell and Robin Bare, US Army Engineer Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX), plant nutritional changes influencing larval development (Wheeler and Center, 1996; Michael Grodowitz, Jan, Freedman, and Dwilette McFarland, unpublished), and high temperatures. While some of these ideas have been studied to an extent, one possible mechanism for low population development has been poorly addressed and only rarely mentioned as a possible mechanism. This is the impact adult nutrition has on reproductive development, egg production, and oviposition rates.

Only minimal information is available on the reproductive development of *H. pakistanae*, let alone adult nutritional requirements. Most of the present literature focuses on its release, establishment, and success as a biological control agent. The first step toward adequately studying adult *H. pakistanae* nutritional requirements is to provide information describing, in detail, basic reproductive morphology in females as well as the overall development of the reproductive system through time. This is an important prerequisite to understanding the influence adult diet has on egg production and oviposition rates. Published accounts are of little help; only one paper describing the female reproductive system exists (Deonier, 1971) and only minimal morphological details are included in this paper.

To address this gap in the literature, the research described here provides details of morphology of the female reproductive system and associated changes through time for *H. pakistanae*. It describes a physiological age-grading system based on changes in the female reproductive system. Such a system will be important for understanding and measuring changes in reproduction in relationship to adult nutritional requirements.

2. Materials and methods

2.1. Source of insects

Rearing methods are modified only slightly from those found in the literature (Freedman et al., 2001). *H. pakistanae* larvae and adults were collected from colonies maintained at the Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, Texas, and from colonies established in the Department of Biological Science at the University of North Texas (UNT) in Denton, Texas. Both colonies consisted of immature rearing containers and oviposition boxes. Rearing containers were 4-l in volume and filled with about 75% hydrilla in water, leaving enough headspace for adults to mate and oviposit. Adults were periodically removed from the containers using an aspirator and sorted by sex and species. Those adults not used for experiments or dissections were placed into the oviposition box to encourage additional ovipositions.

Oviposition boxes were slanted with a height measurement of 54.5 cm (back), 47 cm (front), and a depth of 63.5 cm. The front, measuring 122 cm across, had two circular openings allowing for internal access, each fitted with a cloth sleeve to prevent flies from escaping. The top of the oviposition box was constructed of Plexiglas to allow maximum light penetration. In each oviposition box, small petri dishes with several drops of two adult food mixtures and large petri dishes with hydrilla stems in water for use as an oviposition substrate were included. Immature larvae found on the hydrilla were placed back into the rearing containers to enlarge the colonies. Adult food consisted of a sugar–yeast hydrolyzate mixture (4 g yeast hydrolyzate, 7 g sucrose, 10 ml water) and a sugar–water mixture (1:1) (Freedman et al., 2001). Food was changed frequently to prevent formation of fungal contamination.

Adults for field studies were collected from the surface of experimental ponds containing hydrilla at the LAERF using a hand-held, battery-powered insect vacuum. Flies were collected during the active growing period, i.e., June through September.

2.2. Dissection techniques

Females were dissected alive using a stereomicroscope (25–35×) to prevent deterioration of internal organs that occurs shortly after death. Adults were held individually in plastic vials at room temperature until just prior to dissection. Adults were anesthetized by placing them briefly in a freezer (3–5 min) or by exposure to CO_2. Inactive flies were removed and immediately pinned through the dorsal surface of the thorax; ventral side down, onto an electron microscopy stub covered with a mixture of beeswax, paraffin, and dark-colored crayon wax. The flies were covered completely with a phosphate buffered saline, pH 7 (PBS) in an effort to protect the ovaries from desiccation and deterioration due to osmotic changes. Grasping the abdominal cuticle along the mid-dorsal line with fine forceps and gently tearing the cuticle opened the abdominal cavity, exposing the ovaries. Prior to removal of the ovaries, characteristics of the fat body (if visible) were noted. Ovaries were carefully removed by grasping the common oviduct using fine forceps. The ovaries were subsequently placed onto a microscope slide in a drop of PBS for examination. The ovaries were first viewed using a dissecting microscope, and examined under a compound microscope at 100× and 200× magnifications to distinguish changes in reproductive system morphological markers. Characteris-