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Sense organs on the ovipositor of *Macrocentrus cingulum* Brischke (Hymenoptera: Braconidae): their probable role in stinging, oviposition and host selection process☆

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ABSTRACT

Parasitoid wasps from the insect order Hymenoptera can be deployed successfully as biological control agents for a number of pests, and have previously been introduced for the control of corn pest insect species from the Lepidopteran genus *Ostrinia*. Organs on the ovipositor of parasitoid wasps have mechanical and tactile senses that coordinate the complex movements of egg laying, and the ovipositor of Hymenopteran insects have evolved associated venom glands as part of their stinging defense. The ovipositor of parasitic wasps has evolved an additional function as a piercing organ that is required for the deposition of eggs within suitable host larvae. The morphology and ultrastructure of sense organs on the ovipositor and sheath of *Macrocentrus cingulum* Brischke (Hymenoptera: Braconidae) are described using scanning and transmission electron microscopy. Three types of sensilla trichodea were shown to be abundant on the outer sheath of the ovipositor, with types II and III being most distal, and the inner surface of the ovipositor covered with microtrichia, more densely near the apex. Sensilla coeloconica are distributed on both ventral and dorsal valves, while campaniform sensilla and secretory pores are only located on the dorsal valve. The olistheter-like interlocking mechanism, as well as the morphology of the ventral and dorsal valve tips and the ventral valve seal may be important in stinging, oviposition and in the host selection process.

Introduction

The parasitic wasp *Macrocentrus cingulum* Brischke (Hymenoptera: Braconidae) (syn. *M. grandii* Goidanich) is a polyembryonic endoparasitoid of the Asian corn borer, *Ostrinia furnacalis* (Lepidoptera: Pyralidae) and the European corn borer, *O. nubilalis* (Edwards and Hopper, 1999; Hu et al., 2003). The native range of *M. cingulum* includes most of Europe as well as Asia, where it is distributed in Japan, Korea and China (Watanabe, 1967). Polyembryonic development is advantageous for rapid propagation of a biological control agent, whereby multiple offspring can be produced from a single zygote (Xu et al., 2010). Furthermore, oviposition by *M. cingulum* has been shown to be highly specific for species of the genus *Ostrinia* (Losey et al., 2001), which identified this parasitoid as an ideal candidate for introduction into North America for the control of *O. nubilalis* populations in corn producing areas (Parker, 1931). The efficacy of parasitoid infection of host insects in corn fields has since been determined to range from 4 to 23% in the region (Siegel et al. 1986).

The insect ovipositor is a complex structure associated with the 8th and 9th abdominal segments in females (Dweck et al., 2008). Among the endopterygote insects, members of the order Hymenoptera (bees and wasps) have evolved a well-developed ovipositor apparatus that performs multiple functions. The ancestral function of insect ovipositors is solely for the deposition of eggs onto suitable substrates, which involves the coordination of multiple sensory inputs. The ovipositors of Hymenoptera also have associated venom glands, and ducts for the injection of venom into prey insects, or for use as a defense mechanism, and this additional development required the evolution of unique ovipositor structures. Additionally, parasitic Hymenopteran wasps have specialized sensory organs (Le Ralec et al., 1996) which are required for the location, recognition and acceptance of suitable host insects for oviposition (Papp, 1974). These functions are coordinated by complex physical structures that originate from gonad tissues (Snodgrass, 1931, 1933).

Previous studies of Hymenopteran ovipositor sense organs stylets and sheaths, by scanning and transmission electron microscopy (SEM

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and TEM), have proven useful for defining species-specific morphologies (Le Ralec and Wajnberg, 1990; Brown and Anderson, 1998; Rahman et al., 1998; Vilhhelmsen, 2000; Roux et al., 2005; Dweck et al., 2008; Nacro and Nenon, 2009; Wang et al., 2010; Shah, 2012). Additionally, variation in ovipositor structures has been useful for the classification of parasitoids, as well as for determining phylogenetic relationships (Austin and Field, 1997) and relating ovipositor structure with possible function (Quicke and Fitton, 1995; Quicke et al., 1995; Dweck et al., 2008).

The present study aims to determine the morphology and ultrastructure of sense organs on the ovipositor stylets and sheaths of *M. cingulum* Brischke (Hymenoptera: Braconidae), and to confirm the classification of the different types of sensilla using SEM and TEM. The goal of this research was to better understand the morphology of the sense organs on this structure, which is used in parasitoid host location and oviposition.

Materials and methods

Experimental Insects

The parasitoid wasp, *M. cingulum* Brischke (Hymenoptera: Braconidae) was reared in the laboratory on the host insect, *O. furnacalis*, at a constant 25 °C temperature and a 16:8 light:dark photoperiod as described by Hu et al. (2003). All laboratory procedures were conducted at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China.

Scanning electron microscopy (SEM)

The ovipositor with sheaths was dissected from newly emerged *M. cingulum* parasitoids using a fine forceps, and was placed into a pre-fixation solution (2.5% glutaraldehyde in 0.1 M phosphate buffer solution (PBS), pH 7.4) and incubated at 4 °C for 24 h. Tissues were then changed to a post-fixation solution (1% buffered osmium tetraoxide) for overnight incubation at 4 °C. The specimens were then dehydrated by passage through a series of 30, 50, 70, 80, 90, 95 and 99.9% ethyl alcohol for 1 h each, and were then placed in absolute (100%) alcohol for 15–20 min. The ovipositor stylets were then mounted on stubs with carbon coated double sticky tape and were sputter coated with gold particles in a SPI-Module TM Sputter Coater. Examination and photography were performed using a SEM (JEOL JSM-35) with accelerating voltage set at 6.0 kV. Images were recorded digitally and stored electronically. The ovipositor and sensilla length were measured from digital images using Adobe Photoshop version CS3. At least six ovipositors were investigated, and 18 sensilla of each type were examined to calculate mean values.

Transmission electron microscopy (TEM)

The ovipositor stylets were dissected as described previously and was then placed into a pre-fixation solution (2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2) for 4 h at 4 °C. Fixation took place overnight in fixation buffer 25% glutaraldehyde and 10% acrolein diluted in a 0.1 M cacodylate buffer (pH 7.2). Samples were briefly washed with fixation buffer, placed into post-fixation buffer (1% osmium tetroxide in 0.1 M cacodylate buffer) for 1.5 h and then dehydrated as described previously. After dehydration, the fixed specimens were treated with propylene oxide, and were then transferred to embedding molds with Spur's resin and polymerized in an oven set at 60 °C for 24 h. Ultrathin sections were cut with an ultramicrotome (Reichert, OMU-3) using a diamond blade and were mounted on 50 mesh pioloform coated grids, stained with uranyl acetate and lead citrate, and finally observed in a TEM (JEOL-1200 EX) with accelerating voltage set at 6.0 kV, and images were then recorded digitally and stored on a computer.

Results

Ovipositor morphology

The female genitalia of *M. cingulum* showed that the basic organization is remarkably uniform among the Hymenopteran insects (Rahman et al., 1998). The ovipositors averaged 4.42 ± 0.41 mm in length, and consisted of ventral and dorsal valves and an ovipositor sheath (Fig. 1). When fused together, the internal lumen functions as a passageway for eggs and/or venom.

The paired ventral valves are independent along most of their length, while dorsal valves were fused along their entire length. The tip of the dorsal valve was observed to be enlarged and slightly curved to form a characteristic notch like structure located approximately 1 mm from the distal tip (Fig. 2). The dorsal and ventral valves are interlocked and form a tongue-like structure called the "rachis" (Figs. 2 and 3). The dorsal and ventral valves are parallel with respect to one another, and the former fits into a grove of the latter called the "aulax" (Fig. 3). This interlocking system does not extend to the apex of the ovipositor. Longitudinal thin chitinous flaps form a seal-like structure, which protrudes into the egg canal from the medial ventral portion of each of the lower valves (Fig. 3), and may function as a seal to prevent the loss of egg/venom from the canal.

The single segmented ovipositor sheath was flexible and well sclerotized, with transverse annulations from the base along the entire length, with the exception of the most distal part (Fig. 4). The sensilla trichodea covered the external surface from base to apex with an evenly spaced distribution. Three types of sensilla trichodea were distinctly observed at the apex of the ovipositor sheath. The apex of the ovipositor sheath was pointed and heavily sclerotized, and the inner surface was densely covered with flexible cutaneous microtrichia (Fig. 5). These microtrichia on the ovipositor sheath were unevenly distributed with the highest density at the apex (Fig. 5).

Sensory structures on the M. cingulum ovipositor

Three types of sensilla were identified on the ventral and dorsal valves, as well as on the ovipositor sheath of the *M. cingulum* ovipositor

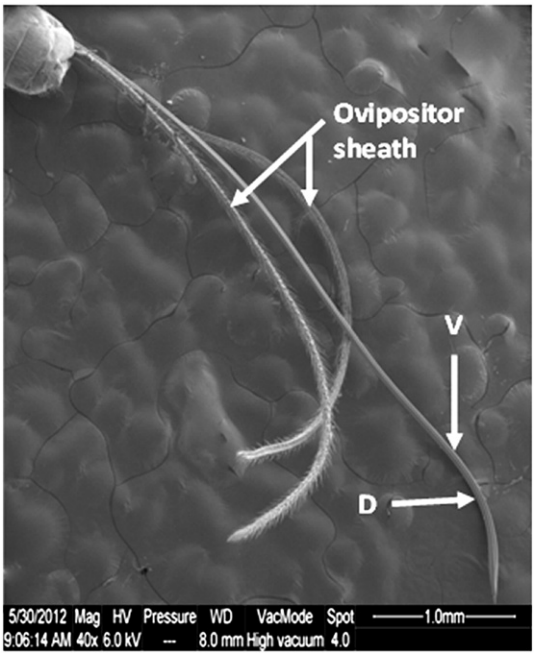

Fig. 1. Ovipositor of *M. cingulum* (total view) 40×; "V" for ventral valve; and "D" for dorsal valve.