Functional anatomy of the vagina muscles in the adult western conifer seed bug, *Leptoglossus occidentalis* (Heteroptera: Coreidae), and its implication for the egg laying behaviour in insects

R.G. Chiang*

Biology Department, Redeemer University College, Ancaster, Ontario, Canada L9K 1J4

ABSTRACT

The anatomy of the female reproductive tract and the nerve-evoked contractions of the vagina muscles and their association with the ovipositor in the western conifer seed bug, *Leptoglossus occidentalis* (Heidemann) are investigated for the first time. The reproductive tract consists of a set of paired telotrophic ovaries, each containing seven ovarioles, located in the anterior lateral regions of the abdomen. Each ovary is attached to a lateral oviduct which spans most of the abdomen to attach to a relatively short common oviduct that joins the vagina near the rear of the animal. The vagina is associated with a pair of bilaterally symmetrical muscles attached at their posterior ends to lateral extensions of sternite VIII, the valvifer of the Type II ovipositor. From this attachment site, the muscles fan out medially and anteriorly to converge along the dorsal midline of the vagina up to the base of the common oviduct. Vagina muscles respond to a single stimulation of their motor nerves by producing a smooth contraction lasting approximately 1 s. With increasing frequencies of stimulation, the muscle contractions summate to create a tetanic response. The muscles are fatigue resistant being able to maintain the same degree of tension for up to 10 min at 10 Hz stimulation. Visual observation shows that other muscles associated with the valves of the ovipositor behave in a similar fashion to that of the vagina muscles from which the tension recordings were obtained. Fatigue-resistant vagina muscles are discussed in relation to copulation, sperm transport and this insect's ability to deposit a series of eggs directly onto the surface of a conifer needle in a manner by which eight or more blunt-ended eggs are packed end-to-end in a single row.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

The ovipositing behaviour of insects is of general interest since insect species have developed a wide variety of egg laying strategies, including the morphological specialization of their ovipositors, to ensure the continuation of the species (for examples, see Salt, 1937; Scudder, 1971; Greany and Oatman, 1972; Hofsvang, 1988; Howlett and Clarke, 2005). Depending on morphology, insect ovipositors are divided into two main types —Type I and Type II (Scudder, 1971). The Type I ovipositor consists of tapered distal abdominal segments which are capable of being retracted in a telescopic manner. Such an ovipositor is usually employed to probe and to deposit eggs deep into crevices or a soft substrate. The Type II ovipositor is not telescoping, and insects with these ovipositors are concerned with depositing eggs in or on firm substrates and tissues. The Type II ovipositor is more widely distributed, shows more morphological variation than Type I (Scudder, 1971), and modifications in its structure are used for taxonomic purposes (see Dolling, 1987; Brailovsky, 2004).

Grasshoppers and locusts possess telescoping Type I ovipositors, and the neuromuscular mechanisms controlling egg laying in these species have been examined (Thompson, 1986a,b; Belanger and Orchard, 1993a,b). The neuromuscular control of a Type II ovipositors is reported by describing the vagina muscle in the blood-feeding hemipteran, *Rhodnius prolixus* (Chiang and O'Donnell, 2009; Chiang et al., in 2010). In this insect, the vagina muscles are attached to sternite VIII, the first genital sternite and valvifer of the ovipositor. Contraction of the vagina muscles not only constricts the lumen of the vagina, it pulls on the valvifer thereby coordinating with egg release. These vagina muscles are capable of smooth contractions with the degree of tension correlated with the frequency of nerve stimulation. This tension is transient and returns to a baseline within 1–2 min during continuous nerve-evoked stimulation. The vagina muscles are also equipped with an inhibitory motor component. Such control over contractions of the vagina muscles would enable this insect to rest the valvifers of

* Tel.: +1 905 648 2139x4241; fax: +1 905 648 2134.
E-mail address: gchiang@redeemer.ca

its ovipositor between the passage of each egg. This suggestion corresponds to the observation that *R. prolixus* deposits eggs in a scattered fashion singly or in batches of two to five side-by-side randomly over the surface of a substrate. Eggs are never seen deposited end-to-end (author's observations).

The present report adds to our understanding of the role of vagina muscles in Type II ovipositors by describing the functional anatomy of the vagina muscles in the western conifer seed bug, *Leptoglossus occidentalis*. This insect resembles the blood-feeding *R. prolixus* in its body size and shape, and in the structure of its ovipositor. Conifer seed bugs suck on the immature seeds of conifer trees. They are known to cause significant damage to the seeds of several species of conifers (Hedlen et al., 1981), resulting in considerable destruction to high-value seed orchards (Bates et al., 2000, 2002; Strong et al., 2001). Unlike *R. prolixus*, *L. occidentalis* lays a string of eight or more blunt-ended eggs end-to-end along the dorsal surface of a single conifer needle. The physiology of their vagina muscles also differs from *R. prolixus* as contractions are fatigue-resistant, and they lack an inhibitory neural input. These attributes are compared with that of *R. prolixus*, and this comparison provides additional insights into the role of the vagina muscles in the Type II ovipositor in these two important insect pests.

2. Materials and methods

2.1. Animals and dissection

Animals used in this study were adult females removed from conifer trees during late summer in Southern Ontario, Canada (August to Sept) or found inside buildings during the fall. They were housed in plastic containers with water and branches with cones from conifer trees, and kept at 20 °C with a 12:12 h light/dark cycle. Under these conditions, some of the captured late larval stages emerged into adults and some of the adult females laid eggs which also hatched. Although the bugs did not survive in the laboratory past mid winter, and a viable laboratory colony has yet to be established, attempts are currently being made to provide a convenient source of these bugs.

To expose the female reproductive tract, the insect was immobilized dorsal side up by placing plasticine over its legs in a plasticine-filled dissecting dish. The dish was then flooded with an insect saline containing 156 mM NaCl, 7.0 mM KCl, 4.0 mM MgCl$_2$·6H$_2$O, 8.2 mM CaCl$_2$·2H$_2$O, and buffered at 7.2 using 20 mM Tris–HCl buffer. The entire dorsal cuticle from the prothorax to the last abdominal segment was removed after cutting the cuticle along its lateral edges in these regions. The oesophagus in the thorax was cut, and forceps used to lift and pull the digestive tract posteriorly out of the body cavity while micro scissors freed the trachea and nerves attaching this tract to the animal. Following removal of the digestive tract, the reproductive system was fully exposed.

2.2. Anatomy

The reproductive tract and its accompanying nerves were visualized with the use of the vital stain methylene blue. After exposing the reproductive system, a few drops of concentrated methylene blue dissolved in insect saline were added to the preparation until the bathing solution became dark blue, but the tissue still visible to the eye. The preparation was left overnight at 5 °C, and the next day, the methylene blue saline was washed away with insect saline. The preparation was intensified and preserved by fixing for 30 min with picric acid adjusted to pH 7 with concentrated NaOH (Stark et al., 1964; Chiang and Davey, 1988). Following several rinses with a saturated aqueous solution of ammonium molybdate, the preparation was examined in the ammonium molybdate. Digital images were captured with a Nikon 8400 camera attached to the photoport of a dissecting microscope equipped with a 2× macro lens, and enhanced with the Adobe Photoshop Elements software. The anatomy was determined by examining more than 20 adult females.

2.3. Tension recordings

Myograms were obtained with the use of an MLT0201 micro force transducer attached by an ML221 Bridge Amp to a PowerLab 2/25 data acquisition system (ADInstruments, Sydney, Australia), and data collected with the Chart 5 software (ADInstruments) (see Chiang and O'Donnell, 2009). The force transducer was mounted on a micro manipulator, and connected to the vagina via a micro hook fashioned from a steel minuten pin. The hook was inserted into the cuticle at the anterior end of the vagina, and attached to the force transducer by a thin plastic thread. To prevent the abdomen from lifting as the vagina contracted, the ventral cuticle was secured to the preparation dish by inserting a minuten pin through the cuticle into the plasticine-filled dish. Stimulating the motor nerves was done through an extracellular suction electrode into which a short portion of the central abdominal connective (abdominal nerve 4) in the thorax was drawn. Square waves of 4 ms duration were delivered by a WPI stimulus isolation unit driven by a WPI Analpulse stimulator (World Precision Instruments, Florida). Threshold voltage was determined by stimulating the nerve at 1 Hz and stepwise increasing voltages until movement of the vagina was observed through the dissecting microscope. To ensure that increasing the frequency was less likely to increase the number of motor axons stimulated in the innervating nerve, voltages approximately 20% above threshold were used to evoke contractions of the vagina muscles. Ten adult females with mature reproductive organs were examined, and shown to display the same response. No differences were detected between females with or without mature eggs in their ovaries.

3. Results

3.1. Ovipositor

The morphology of the ovipositor in *L. occidentalis* is the Type II form described by Scudder (1971). In this form, the most posterior pregenital sternite covers partially the pair of valvifers, which arise from sternite VIII (see Fig. 1A, B). In *L. occidentalis*, sternite VII displays a medial fissure that extends from its posterior edge to midway along the sternite where it ends at a right angle to a minor lateral fold in the cuticle. The valvifers possesses a v-shaped darkly pigmented lateral extension of the cuticle. This extension, which serves as the posterior attachment site for the vagina muscles, is uncovered by removing the overlaying portion of sternite VII (Fig. 1C, D). The valvifer is attached at its ventral anterior end to the base of a valvula. This valvula has the same general shape as the valvifer and forms the medial edge of the ovipositor. It displays darkly pigmented peg-like setae on the posterior half of its ventral margin (see Fig. 1D). Sternite IX covers the anus, and part of it extends ventrally to wedge itself between the posterior ends of the valvifer and valvula of sternite VIII.

3.2. Ovaries, lateral oviducts and eggs

The reproductive tract consists of two ovaries with seven sets of ovarioles. The ovaries occupy only the most anterior lateral regions of the abdomen, and do not extend posteriorly passed the level of sternite II, even in animals undergoing egg production. Attached to each ovary is a lateral oviduct that extends from the base of the ovaries in sternite II to the common oviduct. The common oviduct is relatively short, and connects directly to the vagina at the
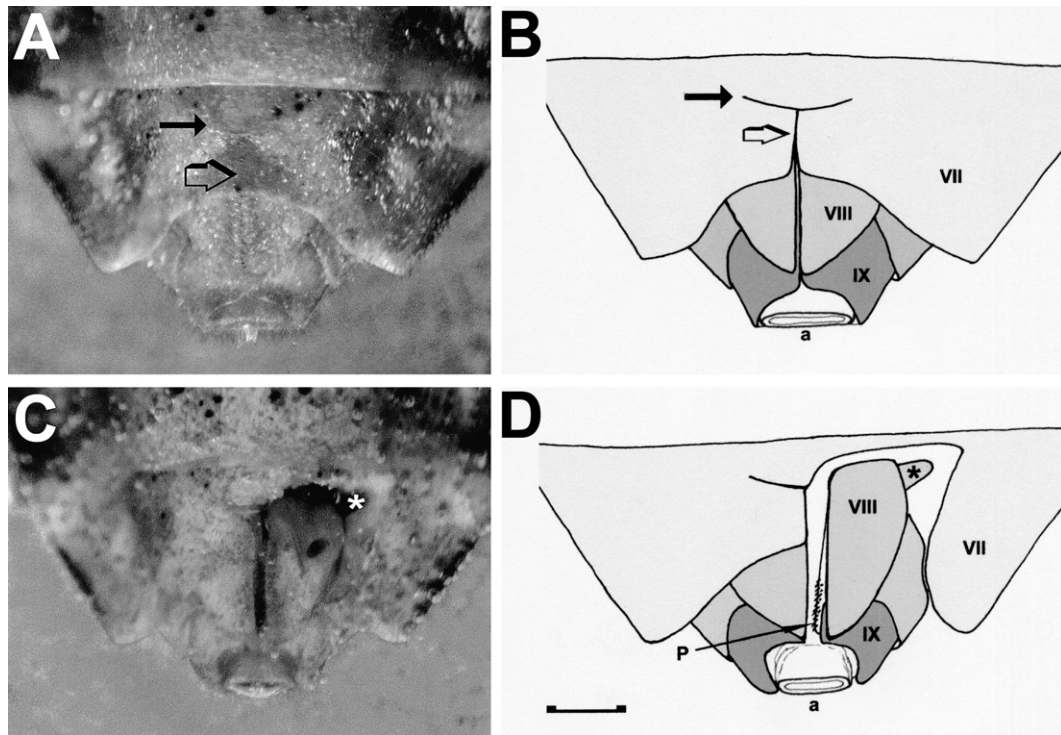
Fig. 1. Photographs and corresponding line diagrams of the ventral posterior region of the abdomen in the western conifer seed bug, *Leptoglossus occidentalis*, describing the Type II ovipositor. A) and B) This exterior view shows the medial cleft or fissure (open arrow) in the most posterior pregenital sternite (VII). The fissure extends from the posterior edge of the sternite to a minor lateral fold in the cuticle (closed arrow) that occurs near the mid horizontal region of the sternite. C) and D) Same preparation as A with the ovipositor slightly extended to show the peg setae (P) on the valvula, and the insertion of sternite IX (IX) between the valvifer (VIII) and the valvula. Part of the left side of sternite VII is removed to expose the lateral extension of the valvifer (asterisk) which serves as the posterior attachment site for the vagina muscles. a, Anus; scale bar, 1 mm.

anterior end of sternite VII. In animals storing mature eggs, the blunt-ended eggs are packed end-to-end along the length of each lateral oviduct (Fig. 2A). Mature eggs are 1.0 mm wide by 1.5 mm long by 0.75 mm high. As many as eight eggs are stored in each lateral oviduct, and to accommodate their total length, which exceeds the length of the abdomen, the oviducts fold onto themselves. The walls of the lateral oviducts are stretched by the eggs they contain, and in live preparations the oviducts contract longitudinally firmly packing the eggs together in a row. When freed from the abdomen of a dissected animal, the force generated by the longitudinal muscles of the lateral oviduct filled with eggs causes the entire oviduct to stretch out into a straight line (Fig. 2A).

Mature eggs are distinguished from the terminal oocytes by their shape and colour. Both possess blunt ends, but the terminal oocytes are white and cylindrical, whereas the mature eggs are brown and D-shaped in cross-section with a flat side. The flat side does not have a particular orientation in the lateral oviducts (Fig. 2A), but becomes ventral as the egg is deposited on the substrate. The oval outline of the operculum, through which the first stage nymph exits the egg, is visible on the dorsal anterior region of the mature egg (Fig. 2B, C). The operculum covers approximately half of the anterior end of the egg whereas the flat side of the egg, which attaches to the substrate during oviposition, possesses a centrally-located elongated oval-shaped indentation (Fig. 2D). In addition to the operculum, the dorsal surface of the egg displays a series of dots running in a curved line from the lateral anterior ventral edge over the posterior region of the operculum forming a shape resembling that of a necklace (Fig. 2B, C).

3.3. Vagina

The vagina is a cuticle-lined epidermal sack that occupies the region from the base of the common oviduct at the anterior end

of sternite VII to the sclerites of the ovipositor (see Fig. 3). From a dorsal view, the vagina is narrowest where it joins the base of the common oviduct, and widest between the lateral extensions of the valvifers. These lateral extensions are curved dorsally shaping sternite VIII into a cup-like structure. Between the lateral extensions, the vagina is pulled relatively tautly, and forms folds running between the lateral extensions. Posterior to these folds, the vagina forms a pouch as it turns ventrally to attach to the anterior edges of the sclerites of the ovipositor. A ring of sclerotized tissue is embedded in the dorsal surface of this pouch.

Attached to the dorsal surface of the vagina between the lateral extensions is the spermathecal complex. This complex is covered from its base on the vagina to the spermathecal pump by the tightly fitting epidermal tissue associated with the vagina. This complex forms a bulging S-shaped structure that lays flat over the dorsal surface of the vagina. It consists of a spermathecal duct, a sperma-thecal dilation, a pump region and the spermatheca. The sperma-thecal complex and its association with a sperm transport system in *L. occidentalis* and Lygaeidae bugs have been recently described (see Chiang, 2009).

The lateral extensions of the valvifers serve as attachment points of three pairs of bilaterally symmetrical muscles with one pair associated with the vagina. The vagina muscle inserts to the posterior end of vagina. The anterior ends of these muscle fibres attach to each other at the midline and in the region posterior to the base of the common oviduct. The other two pairs of muscles are not directly associated with the vagina. The sternal muscle extends ventrally and inserts to sternite VII. It is not visible from a dorsal view. The tergal muscle extends posteriorly and inserts to the apodemes on the dorsal anterior edge of tergite IX. The valvulae are also associated with pairs of muscles. One pair that attaches the anterior edges of the valvula to tergite IX is shown in Fig. 3.
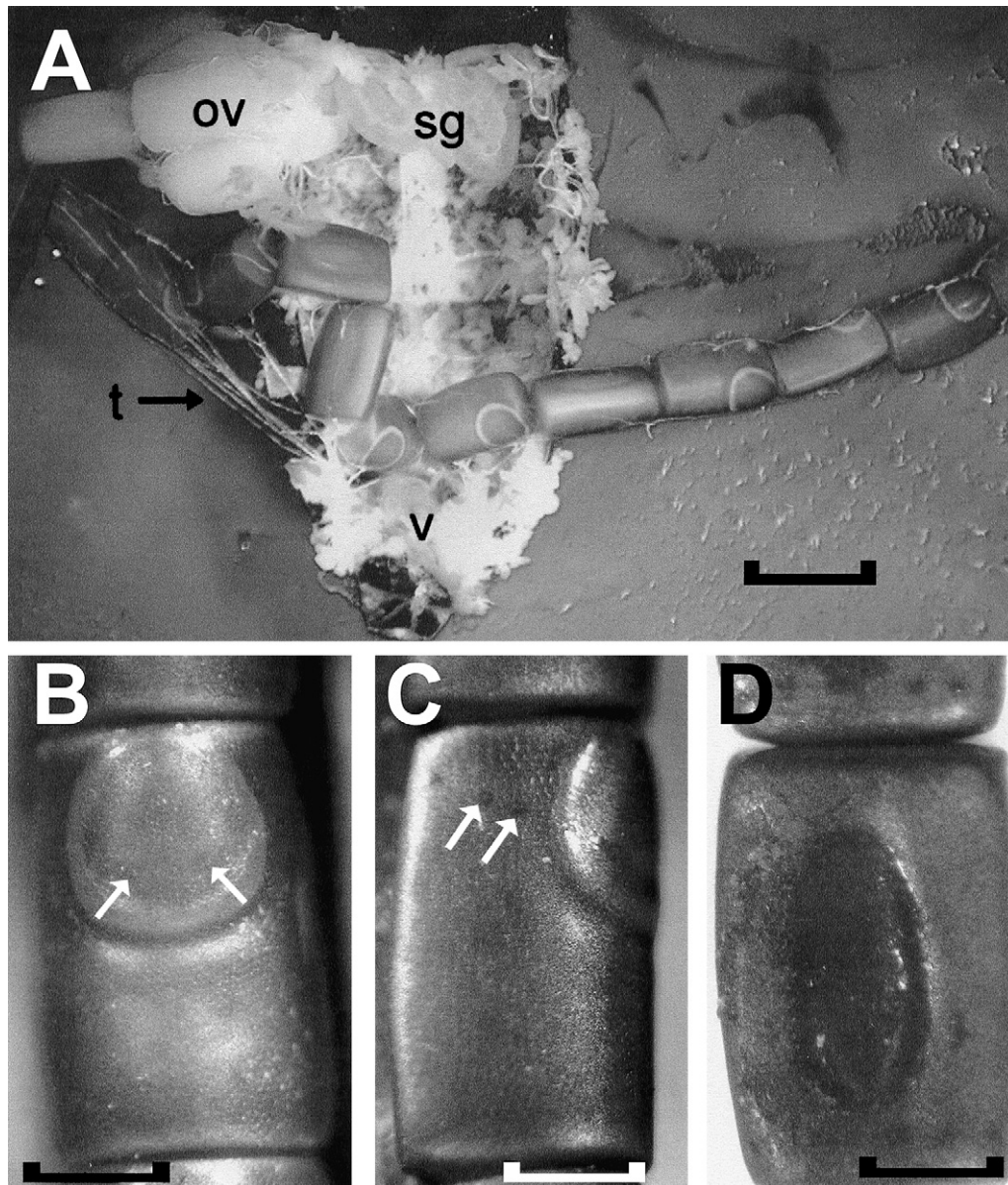
Fig. 2. Female reproductive tract of a living dissected western conifer seed bug, *Leptoglossus occidentalis*. A) Mature eggs in oviducts. Left oviduct is partially folded showing its attachment to its ovary (ov) and tracheal branches (t). Right ovary is free from abdomen allowing its oviduct to be stretched by the firmly packed eggs. sg, Lobes of salivary gland; v, vagina. B) and C) Dorsal and lateral views of a laid egg. Arrows point to dots that are part of the necklace pattern of dots over the surface of the egg. D) Oval indentation on the ventral side of a mature egg lifted from the substrate to which it was attached. Scale bar: 2 mm in A; 0.5 mm in B, C and D.

The anterior end of the vagina joins the common oviduct, and the posterior margins of the vagina (the vulva through which the egg passes onto the substrate) are associated with sternite VII and sternite VIII. The ventral posterior edge of the vagina attaches to the central region of sternite VII along the minor lateral fold. The remaining edges of the vagina attach to the anterior and medial edges of the ovipositor. Much of the folded articulating cuticle is tucked in between sternite VII and sternite VIII, and between the left and right valvifers of sternite VIII. Forcing the posterior end of the vagina open shows that the perimeter of the vulva widens mainly on its ventral side as the folds of articulating cuticle, and the fissure in sternite VII, allow the right and left ventral portions of the ovipositor to separate substantially.

3.4. The nervous system associated with the vagina

The abdomen is supplied by symmetrically arranged nerve branches arising from the fused mesothoracic ganglion in the thorax. Attached to the medial posterior region of this ganglion is a single central connective that extends from this ganglion to the rear of the animal. Just anterior to the common oviduct, nerves that supply sternite VII branch off of this connective. These nerves travel ventral to their respective lateral oviducts, and innervate the left and right posterior ends of sternite VII (see Fig. 3). The central connective continues to the anterior edge of the common oviduct where it divides to give rise to five main nerve branches that travel dorsal to the vagina and serve various parts of the genitalia.

Stimulating the central connective in the thorax and observing movement of the genitalia while cutting different branches reveals that the motor nerves innervating the vagina leave the central connective along the segmental nerves supplying sternite VII. Methylene blue-staining revealed that each of these segmental nerves, shortly after leaving the central connective, gives rise to another nerve branch. This branch turns posteriorly to attach to the wall of the lateral oviduct near its attachment to the common
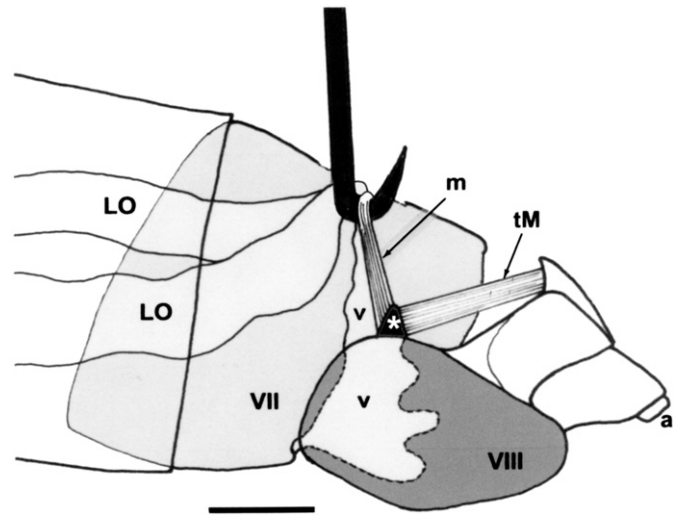
Fig. 3. Diagram of a dorsal view of the vagina and its relation to the nervous system and ovipositor in the western conifer seed bug, *Leptoglossus occidentalis*. For clarity, the nerves dorsal to the vagina are drawn only over the common oviduct, and the structures dorsal to the right valvifer (VIII) of the ovipositor are omitted. A short nerve branches off the nerve that serves the lateral posterior regions of sternite VII fuses with the reproductive tract near the junction of the common and lateral oviducts (arrow head, right side innervation). Each vagina muscle (m) is attached posteriorly to the lateral extension of the valvifer. From this point they extend anteriorly towards the base of the common oviduct fanning out over the surface of the vagina (v). The spermathecal complex, which lays flat over the dorsal surface of the vagina, consists of a spermathecal dilation (D), the spermathecal pump (P) and the spermatheca (s). Open arrow, loop of sclerotized tissue forming a ring on the dorsal surface of the vagina. vM, One of the muscles to the valvula; tM, left tergal muscle attached to lateral extension of the valvifer; LO, lateral oviduct. Scale bar: 1 mm.

oviduct. After removing this branch on one side, only the muscles on the intact side of the vagina contract when the connective is stimulated.

3.5. Vagina muscle contractions

The experimental arrangement to record tension from the vagina muscles is described in Fig. 4, a lateral view of the last abdominal segments. The valvifers of sternite VIII are held firmly to the surface of the dissecting dish by inserting minuten pins through them into the plasticine of the dish. The hook to the force transducer is inserted into the vagina near its junction with the common oviduct, and pulled upwards. Tension generated on the hook during nerve-evoked contractions arises primarily from the vagina muscles, which are in series with the force transducer. The tergal and sternal muscles associated with the valvifers also contract along with the vagina muscles to pull the sclerites together, but little of their tension is recorded by the force transducer.

Following the initial application of tension to the vagina, and prior to evoking contractions by stimulation of the central connective, a slow relaxation of tension is recorded. This relaxation continues for 10–20 min until a steady baseline is reached. This baseline tension is between 100 and 200 mg lower than the initial tension. Very little spontaneous activity is observed, and if present, this activity is infrequent, random and produces a smooth rise and fall in tension similar to the rise and fall of tension seen in evoked contractions (see Fig. 5).

A single stimulus to the innervating nerve elicits a single smooth contraction of the muscle. Increasing the stimulating voltage does not increase the height of these single contractions, whereas increasing frequency does. At 2 Hz stimulation, the tension shows a stepwise increase reaching a plateau after the first few stimuli. As the motor nerve is stimulated with increasing frequencies, the individual contractions summate, and by 10 Hz stimulation, the muscles produce a tetanic contraction (Fig. 6). Increasing frequency

Fig. 4. Diagram of a lateral view of the female genitalia in the western conifer seed bug, *Leptoglossus occidentalis*, showing the anterior end of the vagina muscles hooked in series to the force transducer. The valvifer (VIII) is pinned to the substrate to prevent it from lifting during contraction of the vagina muscles (m). The tergal muscles (tM) also contract with the vagina muscle, but they produce little to no tension on the force transducer. a, Anus; asterisk, lateral extension of the valvifer; LO, lateral oviduct; v, vagina; VII, sternite VII. The appearance of the vagina under VIII is an approximation. Scale bar: 1 mm.

of stimulation increases the rate of rise in tension with the plateau being reached sooner as the frequency is increased from 10 to 30 Hz stimulation. Beyond 30 Hz stimulation, the muscle is less able to reach the maximum degree of tension. At 40 and 50 Hz, the rate of rise in tension is still rapid, but the plateau reached is not increased, and in some preparations, tension shows a gradual decrease during continual stimulation.

The vagina muscles demonstrate the ability to resist fatigue. As seen in the example in Fig. 7, this ability was most evident at 5 and 10 Hz stimulation where the plateau in the tension remains constant for the length of stimulation. At 20 and 30 Hz, the plateau is higher and reached sooner, but the tension gradually falls to a lower level. At even higher frequencies, the rise in tension is still as rapid, but the maximum tension reached is less and the fall in tension during stimulation more obvious. Stimulation can be applied for different lengths of time, and in all cases, tension only dropped to the rest level when the stimulation was stopped.

4. Discussion

The overall appearance of the female reproductive system of *L. occidentalis* bears a number of similarities to previously described

Fig. 5. Tension graphs of spontaneous and nerve-evoked contractions recorded from the vagina muscles of the western conifer seed bug, *Leptoglossus occidentalis*. Top trace illustrates four spontaneous contractions that appeared prior to nerve-evoked contractions at 2 Hz stimulating frequency. Lower traces depict portions of the upper trace that have been expanded to show details of the rise and fall of the spontaneous (left) and nerve-evoked (right) contractions.
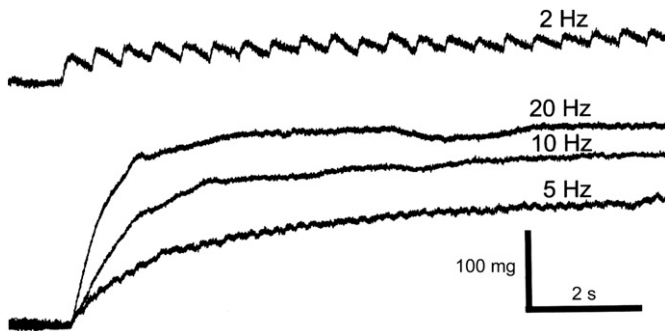
Fig. 6. The initial 10 s of the response of the vagina muscles of the western conifer seed bug, *Leptoglossus occidentalis*, to nerve-evoked stimulation. At 2 Hz (upper trace) the tension of individual contractions was evident. Increasing frequencies to 5, 10 and 20 Hz (superimposed lower traces) caused the contractions to fuse, and the muscles to respond quicker and to reach a higher tension plateau.

Hemiptera (Bonhag and Wick, 1953; Pendergrast, 1957; Gschwentner and Tadler, 2000). It consists of a pair of telotrophic ovaries connected by lateral oviducts to a common or medial oviduct attached to the anterior end of the vagina. As common with seed bugs, the vagina is associated with a spermathecal complex that contains a spermathecal duct, a spermathecal pump and a spermatheca (see Chiang, 2009). A number of other structures characteristic of Type II ovipositors are present, such as the peg setae on the valvifer. Such pegs may represent chemoreceptors important for copulation and egg laying (for example, see Kozánek and Belcari, 2002; Wang et al., in press). The present study was undertaken to expand our understanding of the physiology of vagina muscles of insect Type II ovipositors, and focuses on the structures directly associated with this muscle.
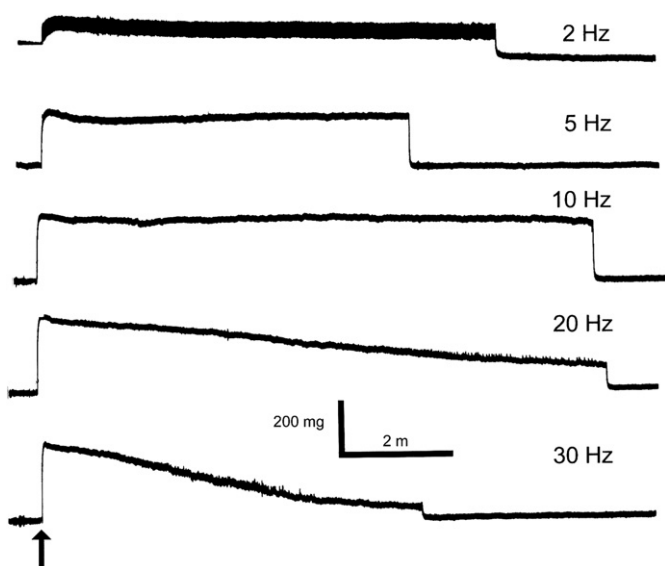

Fig. 7. The long-term (up to 10 min) response of the vagina muscles in the western conifer seed bug, *Leptoglossus occidentalis*, to nerve-evoked contractions delivered continuously at 2, 5, 10, 20 and 30 Hz. Arrow represents the onset of the stimulation. These responses are from the same preparation, with rest periods of 10 min between tests. The broader line of the tension plateau generated at 2 Hz reflects the muscle's ability to relax between stimuli (see 2 Hz stimulation in Fig. 5). In this preparation, 20 and 30 Hz stimulating frequencies could generate rapid strong initial responses, but the muscle could not maintain this tension. At all frequencies, the tensions dropped to baseline when stimulation ceased.

A previous report describes the anatomy and tension development of the vagina muscles in *R. prolixus* (Chiang and O'Donnell, 2009). Although there are many anatomical differences between these two Hemiptera, there are similarities especially within the female reproductive system. A distinct bursa copulatrix is absent. The vagina is a cuticle-lined epidermal sack with bilaterally symmetrical muscles attached to its walls. Contractions of these muscles can also be elicited by stimulating the motor nerves. However, the manner by which the muscles respond to continual stimulation is distinctly different, and these differences may be related to the mechanics associated with copulation, sperm transport and egg laying.

4.1. Copulation

In *R. prolixus*, the contractions of the vagina muscle are more likely to expel the male organ than to secure it in the female. In this insect, the male inserts his intromittent organ into the vagina, and the spermatophore sac at the end of this organ is everted into the vagina. The spermatophore is formed within the posterior end of this organ while it is within the vagina, and when the intromittent organ is removed the spermatophore is left behind (Davey, 1959). Since contraction of the vagina muscles constricts the lumen of the vagina and pull on the valvifers of the ovipositor (Chiang and O'Donnell, 2009), contractions likely serve to expel the egg. Therefore, constriction of the vagina during copulation, which lasts approximately 25 min (Davey, 1958), would likely expel the male apparatus from the vagina rather than securing it in position. In keeping with this suggestion, a myoinhibitor was recently discovered in testes extracts of *R. prolixus*. This myoinhibitor, referred to as rhodtestolin, may be involved in calming the vagina muscles of the female during copulation to reduce the chance of male organ ejection (Martens and Chiang, 2010).

In *L. occidentalis*, a spermatophore is not formed by the male, and the insemination process employs a different mechanism. The slender male processus gonopori enters the opening of the insemination duct at the base of the spermathecal complex, and weaves its way up through the insemination duct to the base of the spermatheca into which it deposits sperm (Gschwentner and Tadler, 2000; Chiang, 2010). Contraction of the vagina muscles constricts the region of the vagina anterior to its connection to the spermathecal complex, and contraction of this muscle alone would not serve to hold the male organ in place. However, the other muscles associated with the lateral extension of the valvifer also respond as the vagina muscle, and the result of continual nerve-evoked stimulation is to pull the sclerites of the genitalia together. Such an action would hold the male organ in the female vagina rather than ejecting it. A mechanism to maintain copulation would be helpful for a species that requires more copulation time for a successful insemination. Although the length of copulation in the western conifer seed bug has yet to be documented, closely related seed bugs need to copulate for as long as 6 h to ensure successful insemination (Micholitsch et al., 2000), a length of time considerably longer than the 25 min needed for *R. prolixus*.

4.2. Sperm transport

The spontaneous and nerve-evoked stimulations of the vagina muscles in these two insects also show similarities and differences. In both, the vagina muscles generate strong smooth contractions of approximately the same maximal level of tension. But in *R. prolixus*, the strong smooth contractions are interspersed with transient short duration peaks in tension, suggesting that the contractions of two types of muscles are being measured (Chiang and O'Donnell, 2009). Such short duration contractions are not observed in *L. occidentalis*. This difference may reflect a difference

in the manner by which sperm are transported to the storage sites in the female. In *R. prolixus*, sperm must be transported from the spermatophore in the vagina to the spermathecae, which are two blind-ended tubes attached to each side of the common oviduct. Davey (1958) showed this transport to depend on rhythmic contractions of the musculature surrounding the opening to the common oviduct, a region referred to as the lip of the vestibulum. The smaller short duration contractions observed in the myograms could reflect the movement of the vestibulum.

In *L. occidentalis*, sperm are delivered directly to the spermatheca by the long processus gonopori of the male and to the oviducts by the sperm transport system (Chiang, 2010). As such, movements of the vagina may not be required to transport sperm to and from the spermatheca. In *Stephanitis pyrioides* (Tingidae), the sperm actually travel well past the vagina up the lateral oviducts to the base of the ovarioles to fertilize the eggs as they are released by the ovaries (Marchini et al., in press). A similar event may be happening in *L. occidentalis* since the mature eggs stored in the lateral oviducts resemble laid eggs in shape and colour.

4.3. Ovipositioning

A second difference in the evoked contractions of the vagina of these insects is the ability of *L. occidentalis* to maintain a steady tension while being stimulated, whereas *R. prolixus* loses this tension within 1–2 min of continual nerve-evoked stimulation. This difference can be related to copulation, as discussed above, or to ovipositing behaviour. *L. occidentalis* lays a string of eggs end-to-end along the dorsal surface of a conifer tree needle. She can be very precise in the deposition of her eggs. Conversely, *Rhodnius* will lay her eggs on any substrate (Schilman et al., 1996), including other members of the colony (author's observations), and although she can retain a large number of eggs in her ovaries, she will lay her eggs individually or side-by-side in small batches. These observations suggest that once *L. occidentalis* finds a location to begin egg laying, this process usually continues until several eggs are laid end-to-end. On the other hand, *Rhodnius* is able to interrupt egg laying, and can scatter her eggs over a substrate. Indeed, nerve-evoked stimulations of the vagina muscles reveal a strong inhibitory component in *R. prolixus* (Chiang and O'Donnell, 2009). At stimulation frequencies of 2 Hz or less, spontaneous contractions are inhibited, and only those frequencies greater than 5 Hz will elicit a contraction. Furthermore, with increasing frequencies of stimulation, the corresponding increase in the initial tension peak is followed by a return to the baseline, a return that can be explained by the effects of a strong inhibitory component. The vagina muscles in *L. occidentalis* do not show this inhibition. A single stimulus will evoke a contraction, and at higher frequencies, tensions can be generated well after the point at which the muscles in *R. prolixus* would have relaxed. Hence, *R. prolixus* is equipped with the motor control well suited for delivering one egg at a time whereas *L. occidentalis* can maintain its vagina muscles in a contracted state possibly to keep the vulva open to allow delivery of eggs one after another without rest periods between eggs. To verify these suggestions an examination of the movements of the vagina and ovipositor during egg laying is warranted.

Acknowledgements

References

Bates, S.L., Lait, C.G., Borden, J.H., Kermode, A.R., Savoie, A., Blatt, S.E., Bennett, R.G., 2000. Impact of feeding by the western conifer seed bug, *Leptoglossus occidentalis* (Hemiptera: Coreidae), on the major storage reserves of mature Douglas-fir (Pinaceae) seeds. Canadian Entomologist 132, 91–102.

Bates, S.L., Strong, W.B., Borden, J.H., 2002. Abortion and seed set in lodgepole and western white pine conelets following feeding by *Leptoglossus occidentalis* (Heteroptera: Coreidae). Environmental Entomology 31, 1023–1029.

Belanger, J.H., Orchard, I., 1993a. The locust ovipositor opener muscle: properties of the neuromuscular system. Journal of Experimental Biology 174, 321–342.

Belanger, J.H., Orchard, I., 1993b. The locust ovipositor opener muscle: proctolinergic central and peripheral neuromodulation in a centrally driven motor system. Journal of Experimental Biology 174, 343–362.

Bonhag, P.F., Wick, J.R., 1953. The functional anatomy of the male and female reproductive systems of the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae). Journal of Morphology 93, 177–283.

Brailovsky, H., 2004. New genera and new species of Colpurini (Heteroptera: Coreidae) from Irian Jaya and Papus New Guinea. Florida Entomologist 87, 566–570.

Chiang, R.G., 2010. A newly discovered sperm transport system in the female of Lygaeidae bugs. Physiological Entomology 35, 87–92.

Chiang, R.G., Davey, K.G., 1988. A novel receptor capable of monitoring applied pressure in the abdomen of an insect. Science 241, 1665–1667.

Chiang, R.G., Martens, J.D., O'Donnell, M.J. The vagina muscles of the bloodsucking insect, *Rhodnius prolixus*, as a model for exploring the physiology of proctolin. Physiological Entomology, doi:10.111/j.1365-3032.2010.00724.x.

Chiang, R.G., O'Donnell, M.J., 2009. Functional anatomy of vagina muscles in the blood-feeding insect, *Rhodnius prolixus*. Arthropod Structure and Development 38, 499–507.

Davey, K.G., 1958. The migration of spermatozoa in the female of *Rhodnius prolixus* Stal. Journal of Experimental Biology 35, 694–701.

Davey, K.G., 1959. Spermatophore production in *Rhodnius prolixus*. Quarterly Journal of Microscopical Science 100, 221–230.

Dolling, W.R., 1987. A mimetic coreid bug and its relatives (Hemiptera: Coreidae). Journal of Natural History 21, 1259–1271.

Greany, P.D., Oatman, E.R., 1972. Analysis of host discrimination in the parasite *Orgilus lepidus* (Hym.: Braconidae). Annals of the Entomological Society of America 65, 377–383.

Gschwentner, R., Tadler, A., 2000. Functional anatomy of the spermatheca and its duct in the seed bug *Lygaeus simulans* (Heteroptera: Lygaeidae). European Journal of Entomology 97, 305–312.

Hedlen, A.F., Yates, H.O., Tovar, D.C., Ebel, B.H., Koerber, T.W., Merkel, E.P., 1981. Cone and Seed Insects of North American Conifers. Canadian Forestry Service, USDA Forest Service and Secretaria de Agricultura y Recursos Hidraulicos, Mexico.

Hofsvang, T., 1988. Mechanisms of host discrimination and intraspecific competition in the aphid parasitoid *Ephedrus cerasicola*. Entomologia Experimentalis et Applicata 48, 233–239.

Howlett, B.G., Clarke, A.R., 2005. Oviposition deterrence is likely an effect, not a mechanism, in the leaf beetle *Chrysophtharta bimaculata* (Olivier) (Coleoptera: Chrysomelidae). Journal of Insect Behavior 18, 609–618.

Kozánek, M., Belcari, A., 2002. The structure of the female postabdomen and associated sensilla of tephritoid flies (Diptera: Tephritoidea). Canadian Journal of Zoology 80, 1389–1404.

Marchini, D., Del Bene, G., Dallai, R. Functional morphology of the female reproductive apparatus of *Stephanitis pyrioides* (Heteroptera, Tingidae): a novel role for the pseudospermathecae. Journal of Morphology, doi:10.1002/jmor.10811.

Martens, J.D., Chiang, R.G., 2010. Testes extracts inhibit heart contractions in females of the blood-feeding insect, *Rhodnius prolixus*. Insect Sci, doi:10.1111/j.1744-7917.2010.01317.x.

Micholitsch, T., Krugel, P., Pass, G., 2000. Insemination and fertilization in the seed bug *Lygaeus simulans* (Heteroptera: Lygaeidae). Eur J Entomol 97, 13–18.

Pendergrast, J.G., 1957. Studies on the reproductive organs of the Heteroptera with a consideration of their bearing on classification. Transactions of the Royal Entomological Society of London 109, 1–63.

Salt, G., 1937. The sense used by *Trichogramma* to distinguish between parasitized and unparasitized hosts. Proceedings of the Royal Society of London 122, 57–75.

Schilman, P.E., Nunez, J.A., Lazzari, C.R., 1996. Attributes of oviposition substrates affect fecundity in *Rhodnius prolixus*. Journal of Insect Physiology 42, 837–841.

Scudder, G.G.E., 1971. Comparative morphology of insect genitalia. Annual Review of Entomology 16, 379–406.

Stark, M.J., Smalley, K.W., Rowe, E.C., 1964. Methylene blue staining of axons in the ventral nerve cord of insects. Stain Technology 44, 97–102.

Strong, W.B., Bates, S.L., Stoehr, M.U., 2001. Feeding by *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) reduces seed set in lodgepole pine. Canadian Entomologist 133, 857–865.

Thompson, K.J., 1986a. Oviposition digging in the grasshopper. I. Functional anatomy and the motor program. Journal of Experimental Biology 122, 387–411.

Thompson, K.J., 1986b. Oviposition digging in the grasshopper. II. Descending neural control. Journal of Experimental Biology 122, 413–425.

Wang, X.-Y., Yang, Z.-Q., Gould, J.R. Sensilla on the antennae, legs and ovipositor of *Spathius agrili* Yang (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). Microscopy Research and Technique, doi:10.1002/jemt.20795.