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Functional anatomy of female sex pheromone gland of *Mayetiola destructor* Say (Diptera: Cecidomyiidae)

ABSTRACT

FUNCTIONAL ANATOMY OF FEMALE SEX PHEROMONE GLAND OF *MAYETIOLA DESTRUCTOR* SAY (DIPTERA: CECIDOMYIIDAE)

The Hessian Fly (*Mayetiola destructor* Say, Diptera Cecidomyiidae) sex calling of males by females, known from long ago, has been recently demonstrated of being mediated by a female produced sex pheromone that has been chemically identified lately.

Following the investigation methods previously applied to study sex pheromone glands in other Cecidomyiidae species (i.e., *Allocontarinia sorghicola* (Coq.) Solinas and *Dasineura brassicae* Winn.), the female sex pheromone gland of the Hessian Fly has been identified through functional-anatomy, ultrastructural, and physiological observations here reported. The pheromone gland consists of the 8th-9th abdominal intersegmental membrane transformed epidermis. This appears quite different in virgin than mated females being in the former strongly hypertrophied and obviously atrophied in the latter.

Key words (in addition to those in title): Gross anatomy, histology, insect, midge, ovipositor, physiology, ultrastructure.

INTRODUCTION

The Hessian fly (*Mayetiola destructor* Say), an originally palaearctic species (probably from Europe) then diffused all over Europe, Middle East, North Africa, North America (from coast to coast), Australia and New Zealand (ROBERTI, 1957; SKUHRAVÀ, 1986; GAGNÉ, 1989), is a major pest of wheat (*Triticum aestivum L.*) and also capable to develop on rye (S*ecale cereale L.*), barley (*Hordeum vulgare L.*), *Elymus sp.,* and possibly other grasses as accidental hosts (GAGNÉ, l.c.).

The economic importance of the Hessian fly, combined with lack of efficient, traditional control measures, poses a serious crop protection problem thus far only partially resolved by means of resistant wheat varieties (especially in U.S.A.).

The ecology and behaviour of the adult midges (CARTWRIGHT, 1922; MCKAY and HATCHETT, 1984; HARRIS and FOSTER, 1991) suggest that the use of female

sex pheromones for the Hessian fly biorational control may be a realistic possibility. In fact, as in most Cecidomyiidae, the adult midges live for few days only, accomplishing a single reproductive cycle: just after emergence, the females start a peculiar sex calling behaviour (i.e., they repeatedly extend and retract the ovipositor, waving it back and forth) and thereby conspecific males are attracted to females by a volatile pheromone (HARRIS and FOSTER, 1991). Males copulate several times, whereas females are strictly monogamous due to factors injected by the male during copulation that induce refusal of further mating and stop sex pheromone biosynthesis in mated females (FOSTER *et al.*,1991b; BERGH *et al.*,1992).

The Hessian fly female sex pheromone major component has been isolated from excised ovipositors and chemically identified as (2S)-(E)-10-tridecen-2-yl acetate (FOSTER *et al.,* 1991a).

Our aim was to contribute to a fuller understanding of the Hessian fly chemical ecology (e.g., facilitating the whole pheromone collection for its complete identification) and biorational control (e.g., helping applied entomologists prepare appropriate pheromone traps and/or pheromone dispensers to manipulate the midge behaviour in the field) through the anatomical identification, with histological and ultrastructural illustration and physiology of the gland producing the pheromone(s) in question.

MATERIALS AND METHODS

Insects were from puparia kindly supplied by Dr. M.O. Harris from Kansas (USA), and reared in a bioclimatic chamber at 22-23°C, 60% RH and under 16 h light, 8 h dark photo period. At emergence, adult midges were sexed and males and females were placed in separate cages until they were used.

For functional anatomy studies, CO_2 anaesthetised females were dissected and their excised abdomens and/or ovipositors were studied in fresh whole mounts in 0,9% saline and/or after preparation according to GISIN'S (1960) method, and finally photographed through a Zeiss III Photo microscope.

For transmission electron microscopical studies, just emerged or 24h old virgin females and mated ones nearly 25h old (24h after copulation) were sacrificed through immediate immersion in KARNOVSKY'S (1965) fixative. Their ovipositors were excised, kept in the same fixative for 3 h at 4 °C and washed overnight in cacodylate buffer. The specimens were postfixed in 1% osmium tetroxide in cacodylate buffer for 1 h, rinsed in the same buffer, dehydrated in a graded ethanol series and embedded through propylene oxide in Epon-Araldite. Thin sections, cut with an L.K.B. "Nova" ultramicroto-

me, were sequentially stained with uranyl acetate and lead citrate and examined through a Philips EM 400T.
For scanning electron microscopical observations, newly emerged females, anaesthetised in CO_2, were immersed in 50% ethanol water solution overnight at 4 °C and dehydrated in a graded ethanol series. They were critical point dried in a Balzers Union CPD 020 unit, gold coated in a Balzers Union SCD 040 sputter unit, and viewed and photographed through a Philips XL 20 electron microscope.

Symbols used in the Figures:

CB: cell boundaries; CUe: epicuticle; CUp: procuticle; GR: groove(s); H: hemocoel; IM: intersegmental membrane between 8th-9th uromeres; LV: lysosome-like vesicles; LY: lysosome(s); M: mitochondria; MI: microtrichia; MU: muscle(s); OV: ovipositor; P: minute protuberance(s); PG: pheromone gland; PR: proctodeum; R: ribosomes; SEc: smooth endoplasmic reticulum of cisternal type; SEt: smooth endoplasmic reticulum of tubular type; SJ: septate junction(s); TS: tactile setae; V: secretion vesicle(s); VA: vagina; VIII, IX, X: 8th, 9th, 10th uromeres, respectively; VIIIr: retractile portion of the 8th uromere.

RESULTS AND DISCUSSION

Ovipositor gross anatomy

The *M. destructor* ovipositor is of the Kieffer's (1900) "à pochette" type, i.e., a telescopic one consisting of the 8th + 9th + 10th abdominal segments (fig. 1a, b, VIII, IX, X.). The 8th uromere shows two well distinct portions: an anterior, having the outer likeness of the preceding segments, and a posterior, tubular, entirely membranous and retractile portion bearing tactile setae (fig. 1c, TS) randomly distributed. The 9th uromere is almost as long as the 8th in which it slides during extension/retraction (fig. 1b). A sleeve-like intersegmental membrane (fig. 1b, IM), as long as the 8th uromere tubular portion, connects this latter to the 9th segment allowing it to be telescoped for oviposition or calling behaviour and to be completely retracted in resting position. The 10th abdominal segment is made of two fleshy lamellae: a superior one, the 10th urotergum (also interpreted as fused cerci), and an inferior one, the 10th urosternum.
The ovipositor has been proven to be the source and releasing site of the sex pheromone through previous behavioural bioassays (see introduction),and the above mentioned intersegmental membrane (fig. 1b,

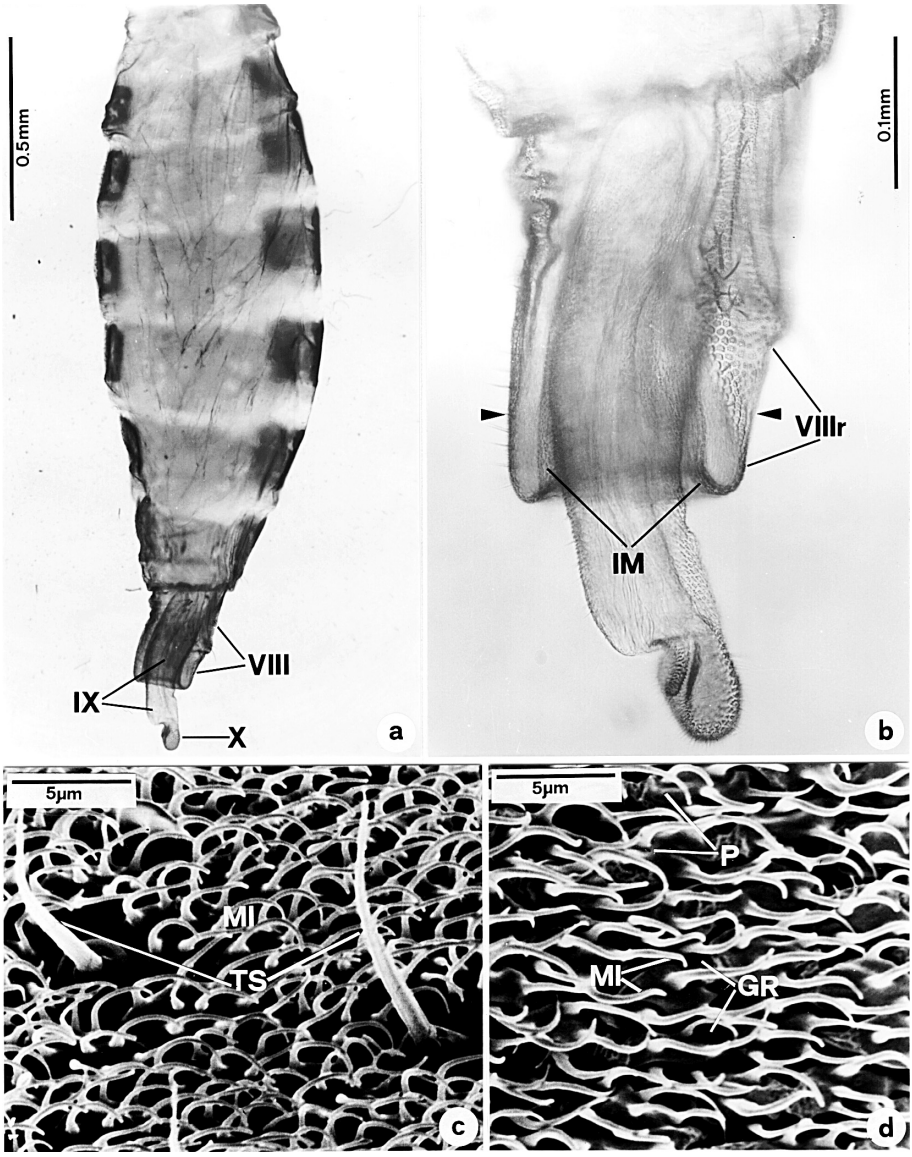

Fig. 1 - *Mayetiola destructor* Say female: (a) whole abdomen displaying ovipositor partially extended (left side), (b) caudal detail of the same, showing 8th uromere almost completely protruded while the 8th-9th intersegmental membrane (IM) is still retracted and sheathing about 2/3 of the 9th uromere (IX); (c) and (d) S.E.M. micrographs displaying outer details of the retractile portion (VIIIr) of the 8th uromere and the intersegmental membrane, respectively.

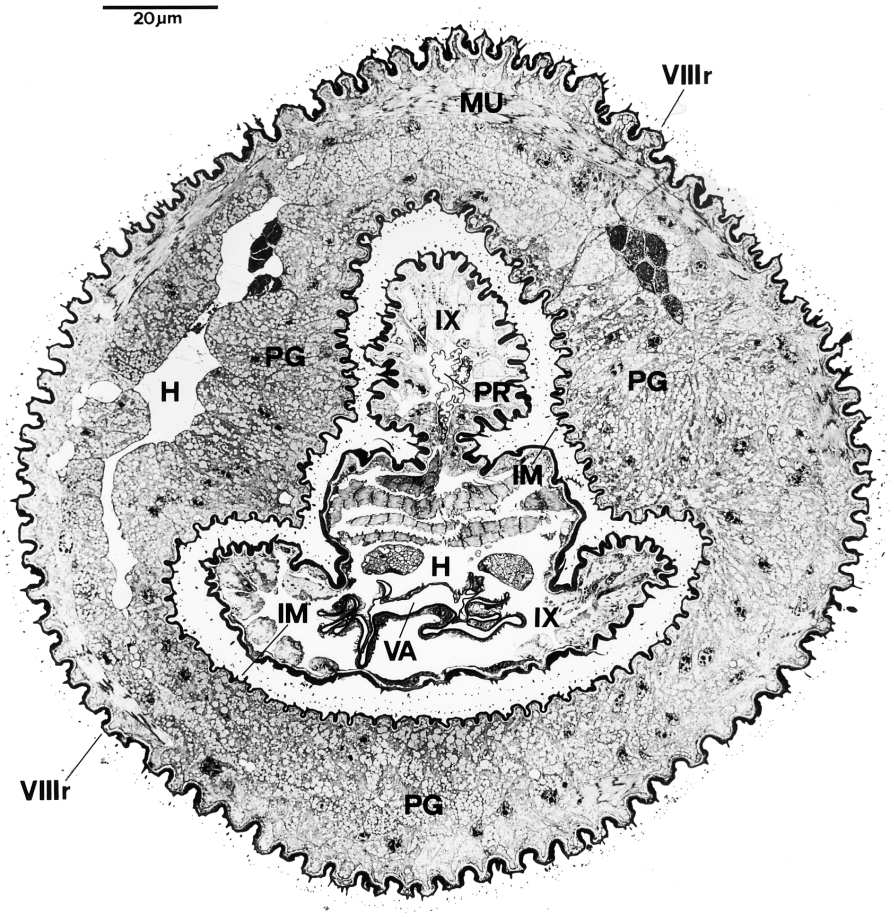

Fig. 2 - Newly emerged virgin female, ovipositor cross section at the level indicated in Fig. 1b (arrow heads). Note the great development of the intersegmental membrane (IM) epidermis (i.e., the pheromone gland: PG) all around.

IM) epidermis is the unique secretory tissue present in the ovipositor hence the unique candidate for being the pheromone gland. The same histological condition has been previously found in the ovipositor of the sorghum midge (Solinas and Isidoro, 1991) and the brassica pod midge (Isidoro *et al.*, 1992).

Gland histology, ultrastructure and physiology

The midge virgin females, both newly emerged or 24h old (at least), display the 8th-9th abdominal intersegmental membrane epidermis (i.e.,the above presumed pheromone gland, fig. 2, PG) hypertrophic and consisting of

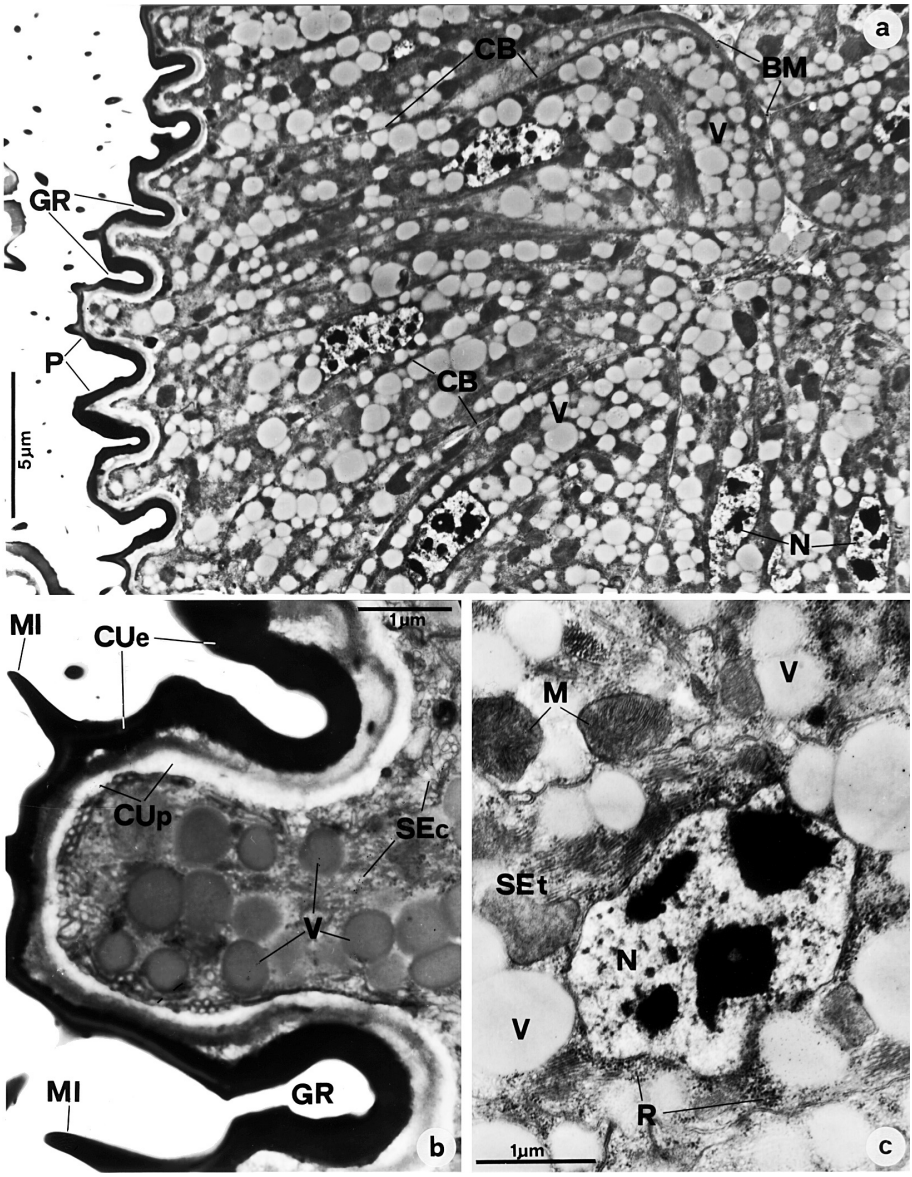

Fig. 3 - Newly emerged virgin female. Details of pheromone gland cross section showing: (a) group of secretory cells bearing striking abundance of secretion vesicles (V) everywhere (i.e., the most conspicuous element of the cells); (b) secretory cell apical portion within a “minute protuberance” (note the great difference in thickness of epicuticle (CUe) on the grooves and the protuberance distal end); (c) nucleus and perinuclear region of a secretory cell.

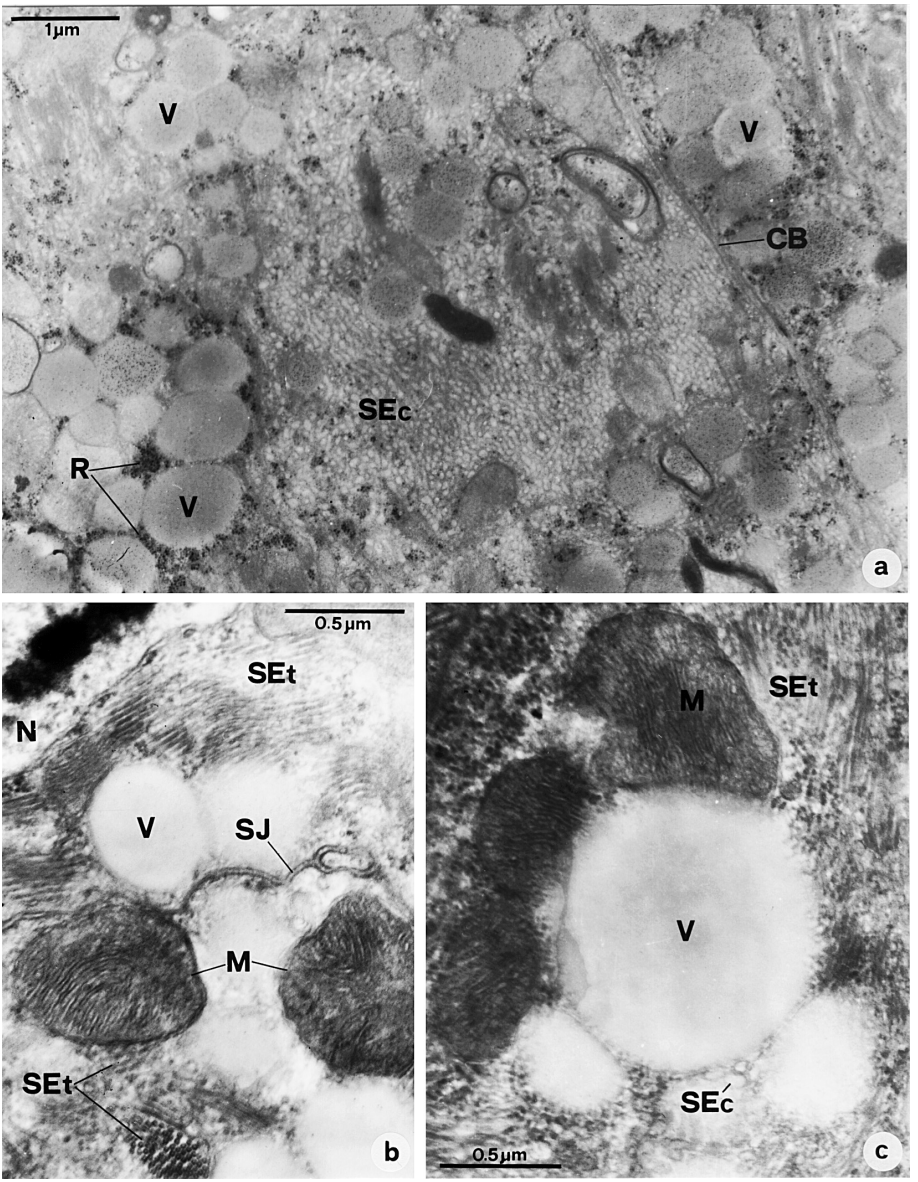

Fig. 4 - Newly emerged virgin female. Secretory cell details showing: (a) cisternal smooth endoplasmic reticulum (SEc) among numerous secretion vesicles (V) near the basal region; (b) tubular smooth endoplasmic reticulum (SEt) and mitochondria (M) with whorled cristae; (c) mitochondria and smooth endoplasmic reticulum (both tubular and cisternal type) surrounding a big secretion vesicle (V).

a single layer of cylindrical-cuboidal, apparently secretory cells of the Noirot and QUENNEDEY'S (1974) first class, resting on a well-developed, rather thick basement membrane (fig. 3a, BM). These cells show a quite even basal plasma membrane, while the apical plasma membrane (fig. 3b) is moderately convoluted, (like in *Allocontarinia sorghicola,* SOLINAS and ISIDORO, 1991, and in *D. brassicae,* ISIDORO *et al.*, 1992). The lateral cell boundaries, as usual in epidermal tissue, frequently display septate junctions (fig. 4b, SJ) towards the apical part. The cells in question possess a typical secretory cell nucleus, and cytoplasm containing: very extensive smooth endoplasmic reticulum of 2 different types, i.e. cisternal and tubular; a small amount of granular reticulum confined around the nucleus; abundant ribosomes (figs 3c, 4a, R) isolated or in groups randomly distributed throughout the cell; well-developed mitochondria (figs 3c, 4b, c, M) frequently exhibiting whorled cristae; Golgi apparatus not so evident as in the above mentioned Cecidomyiidae species; extremely abundant, very varied in size, more or less electron dense secretion vesicles (figs 3, 4, V), as a whole representing the most conspicuous element of the cell.

It is worth remarking that features such as tubular and cisternal smooth endoplasmic reticulum have been interpreted (LALANNE-CASSOU *et al.*, 1977) as reflecting the presence of different biosynthetic pathways in pheromone-producing cells. HALLBERG and RESCHKE (1990) in *Dendrolinus pini* L., whose principal pheromone constituent is an aldehyde (PRIESNER *et al.*, 1984), have found the pheromone-producing cells displaying smooth endoplasmic reticulum of the tubular type; while PERCY (1975) in *Orgyia leucostigma* Hb., which has a pheromone of several compounds including a ketone, has found mainly cisternal but also tubular reticulum in the cells in question; and FILSHIE and WATERHOUSE (1968) in *Nezara viridula* L. lateral scent gland, which secrete a mixture of compounds with the major constituent being an acetate, have also found out cisternal smooth endoplasmic reticulum being prevalent in the secretory cells. In our instance, the pheromone identified is a blend with an acetate as major component (HARRIS and FOSTER, 1991) and we have found smooth endoplasmic reticulum of both the cisternal and tubular types in the pheromone-producing cells.

The cuticle overlying the cells does not show any obvious porosity, and consists of a thick, electron lucid procuticle (fig. 3b, CUp), most of it looking endocuticle, and an electron dense epicuticle (fig. 3b, CUe), remarkably varied in thickness being much thicker on the grooves (fig. 3a, b, GR) and comparatively thin on the "minute protuberances" (fig. 3a, b, P).

At low magnification, the outer surface of the pheromone gland bearing

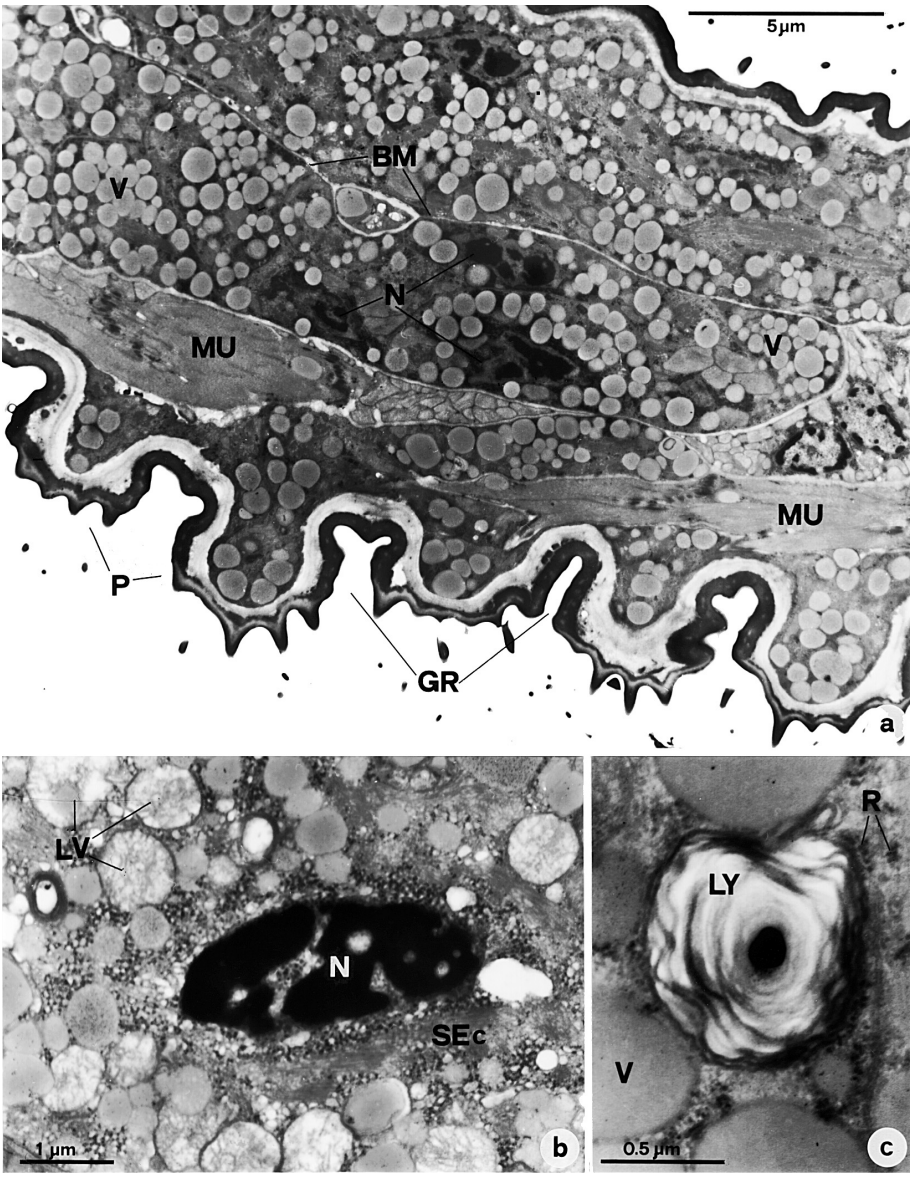

Fig. 5 - 25h old, mated female sacrificed 24h after copulation. Details of pheromone gland cross section, at the level indicated in Fig. 1b (arrow heads), showing: (a) secretory cells obviously atrophied, while secretion vesicles (V) are still abundant but most of them appear undergoing content transformation; (b) and (c), secretory cell details displaying nucleus apparently pychnotic (N) and lysosomes (LY).

membrane (fig. 1b, IM) looks almost smooth but at higher magnification it appears exhibiting continuous series of alternate, irregular, minute protuberances (figs 1d, 3a, 5a, P) and grooves (figs 1d, 3a,b, 5a, GR), the former bearing few short microtrichia (figs 1d, 3b, MI). These outer features may form a special device capable of storing the pheromone and regulate its releasing during the ovipositor extension by the sex calling female midge.

Mated females, sacrificed 24h after copulation, display the secretory epithelium in question much thinner (i.e., less than half thickness as a whole) than in virgin ones of same age, and having gland cells (fig. 5a) obviously atrophied (compare fig. 3 with fig. 5) and showing nucleus apparently pycnotic (fig.5b), although the secretion vesicles (fig. 5a, V) appear still numerous but most of them exhibiting some modification in their content (fig. 5b, LV; crinophagy ?).

This atrophy of the sex pheromone gland in mated females accords with the observations which prove that mating results in the cessation of Hessian fly calling behaviour and pheromone biosynthesis (FOSTER *et al.* 1991b) due to the transfer of chemical factors from the male to the female during insemination (BERGH *et al.*, 1992).

CONCLUSIONS

Knowing the Hessian fly mating behaviour being mediated by a female sex pheromone blend of which only one component (most probably the major) has been identified thus far, our aim was to contribute to a fuller understanding of the midge chemical ecology and its biorational control through anatomo-functional identification of the pheromone-producing gland.

Our results and relative discussion allow the following conclusions:

1. The Hessian fly ovipositor (8th + 9th + 10th abdominal segments), known as the source and the releasing site of the female sex pheromone, contains the pheromone-producing gland.
2. The pheromone gland consists of the 8th-9th abdominal intersegmental membrane epidermis. It appears quite different in virgin than mated females being in the former strongly hypertrophied and apparently atrophied in the latter.
3. The secretory cells exhibit ultrastructural features, such as very extensive smooth endoplasmic reticulum and numerous mitochondria with whorled cristae, very typical of pheromone-producing cells.
4. The presence of smooth endoplasmic reticulum of both the cisternal and tubular types might confirm that the Hessian fly sex pheromone consists of a blend including an acetate.

5. The most conspicuous element of the cells in question is an enormous abundance of secretory vesicles in the virgin females, that do not completely disappear in the hypotrophic cells of the mated females even 24h after copulation, i.e., when most of the latter, in nature, have accomplished oviposition and die.

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RIASSUNTO

ANATOMIA FUNZIONALE DELLA GHIANDOLA A FEROMONI SESSUALI DI *MAYETIOLA DESTRUCTOR* SAY (DIPTERA: CECIDOMYIIDAE)

Il richiamo sessuale dei maschi da parte delle femmine della Cecidomia distruttrice del grano (*Mayetiola destructor* Say, Diptera Cecidomyiidae), noto da lungo tempo, è stato recentemente dimostrato esser mediato da un feromone sessuale il cui composto principale è stato anche chimicamente identificato ultimamente.

Seguendo lo stesso metodo messo a punto dagli stessi autori per lo studio delle ghiandole a feromoni di altre cecidomie (*Allocontarinia sorghicola* (Coq.) Solinas e *Dasineura brassicae* Winn.), è stata identificata la ghiandola a feromoni sessuali della Cecidomia distruttrice del grano, mediante indagini anatomo-funzionali, ultrastrutturistiche e fisiologiche qui riportate. Detta ghiandola a feromoni risulta costituita dall'epidermide trasformata della membrana intersegmentale tra 8° e 9° urite, la quale si presenta fortemente ipertrofica nelle femmine vergini sessualmente mature, mentre si riduce bruscamente di volume e si atrofizza in seguito all'accoppiamento.

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