Boll. Ist. Ent. «G. Grandi» Univ. Bologna, 47: 27-48, 1992

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Mating behaviour and identification of the female sex pheromone gland in the brassica pod midge (*Dasineura brassicae* Winn.: Cecidomyidae, Diptera) (*) (1) (2)

INTRODUCTION

The Brassica Pod Midge (*Dasineura brassicae* Winn.), a typical medium sized *Dasineura* species widely distributed in Europe, is a pest of oilseed rape (*Brassica napus* L.) and other *Brassica* crops (Bromand, 1990). Its larvae develop gregariously inside the siliquas which consequently become misshapen, discoloured and often turn yellow and open prematurely preventing normal seed production. Because the membranous ovipositor of genus *Dasineura* is incapable of piercing *Brassica* pods, the female midge usually oviposits through the holes made by the cabbage seed weevil (*Ceutorhynchus assimilis* Payk.) for feeding and oviposition, although she is not entirely dependent on previous damage done to pods by other insects for successful oviposition (Stechmann & Schuette, 1978).

The economic importance of the seed weevil alone is not usually great but the combined infestation of *C. assimilis* and *D. brassicae* poses a serious crop protection problem, as control measures appropriate for both insects are lacking.

The ecology and behaviour of the adult midges (Sylvén, 1970; Petterson, 1976; Williams *et al*, 1987a & b) suggest that the use of semiochemicals for control may be a possibility. Females of *D. brassicae* produce a sex pheromone probably from the ovipositor (Williams & Martin, 1986). To date, sex pheromones or sex pheromone glands have only been identified in two Cecidomyiids, the Hessian Fly (*Mayetiola destructor* Say, Foster et al., 1991a & b) and the Sorghum Midge

(*) Accepted for publication July 28, 1992.

(1) This is a joint project reflecting individual competence and the results of discussions. However, anatomical and ultrastructural studies are by Isidoro and Solinas, behavioural studies are by Williams and Martin.

(2) Research financially supported by Italian National Council for Research (CNR, 90.02740.CT06) and Italian Ministry for University and Scientific & Technological Research (MURST 60%).

(*Allocontarinia sorghicola* Coq., Solinas & Isidoro, 1991).

The present paper reports investigations into mating behaviour of *D. brassicae* and identifies the sex pheromone gland through fine anatomo-physiological studies.

Material and Methods

Insects

On 10 June, field collected winter rape plants containing *D. brassicae* larvae were placed with their stems in water and their pods over a tray of soil, in an outdoor insectary. Mature larvae left the pods, dropped to the soil and pupated within it. Cocoons, recovered from the soil during September-December by washing it through sieves, were placed in glass vials (10 per vial) on moist sand (20 mm deep) and covered with further moist sand (2 mm deep). They were kept in diapause at 5°C for 150-200 days before transfer to a controlled environment cabinet maintened at 18°C and LD 16 : 8 h cycle, where adult emergence commenced 10 days later.

Mating behaviour observations

On emergence, virgin female (n = 15), male (n = 11) and paired virgin female and male (n = 13) *D. brassicae* were each placed into glass vials (75 x 30 mm diam.) containing moist sand (20 mm deep) and closed with a cotton wool plug. Vials of females and pairs were placed in one incubator and those of males in another; both incubator were at 18°C on a LD 16: cycle synchronous with that of the emergence room. Midge behaviour was recorded at 30 min intervals from 2 h 15 min until 15h 45 min after the onset of photophase, each day until death of individuals or one of a pair. It was noted whether females were resting, walking, "calling" or walking with ovipositor fully extended, or resting or walking with ovipositor partly extended, and whether males were flying, walking or resting. Copulation was also observed.

Anatomy and ultrastructure

For anatomo-functional studies, CO_2 anaesthetized 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. For histology and trasmission electron microscopical studies, virgin females (newly emerged and 24 h old) and mated ones nearly 25 h old (24 h after copulation) were immediately immersed in Karnowsky's (1965) fixative. Their ovipositors were excised and 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, rinced in the same buffer, dehydrated in a graded ethanol series and embedded through propylene oxide in Epon-Araldite. Thin

(gold to silver) sections, cut with an L.K.B. "Nova" ultramicrotome, were sequentially stained with uranyl acetate and lead citrate and examined through a Philips EM 400T. For scanning electron microscopical observations, newly emerged females, anaesthetized 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 were viewed and photographed through a Philips 501 B electron microscope.

Symbols used in the Figs.

BM	basement membrane
CB	cell boundaries
CU	cuticle
G	Golgi apparatus
GR	groove(s)
H	hemocoel
IM	intersegmental membrane between 8th-9th uromeres
LY	lysosomes
M	mitochondrion
MI	microtrichia
MU	muscle(s)
N	nucleus
OC	ovipositor outer cavity delimited by the folded intersegmental membrane (IM) outer surface
ON	ovipositor nerve
OV	ovipositor
P	minute protuberance(s)
PG	pheromone gland
PR	proctodeum
R	ribosomes
SE	smooth endoplasmic reticulum
SJ	septate junctions
TS	tactile sensilla
V	secretion vesicle(s)
VA	vagina
8,9,10	uromeres eighth, ninth, tenth
8C	pocket formed by a retracted part of the 8th uromere tubular portion and the intersegmental membrane

Results and Discussion

Mating behaviour

Female *D. brassicae* survived and were observed for a mean of 4.7 ± 0.34 days, males for a mean of 3.5 ± 0.41 days and pairs were observed for a mean of 3.8 ± 0.31 days. Observations made on the day of death were not included in the analyses of behaviour as the midges were inactive on that day.

Isolated virgin females spent most of their time either resting or walking with the ovipositor retracted (Fig. I, a). However, each female (n= 15) also frequently extended her ovipositor fully in a strong upward curve behind her and waved its tip (9th & 10th uromeres, see Fig. II, 1) about slowly (see Fig. XIV, 1) usually while stationary. The wings were folded at rest over her back during this "calling" behaviour. Most "called" on more than one day; of the total of 56 female midge days observed, "calling" was recorded on 37 days. "Calling" was most intense on the second day of life; the percentage of females that "called" on different days were day 1, 53%; day 2, 93%; day 3, 54%; day 4, 71%; day 5, 75%; day 6, 0%. "Calling" was usually performed while hanging upside down from the cotton wool plug, and was intermittent through the day. Bouts appeared to differ in length; of

a total of 71 observations of "calling", 51% were seen on one observation only and not on the next, 47% were seen on 2-6 consecutive occasions, 1% on 9 and 1% on 13, and therefore may have lasted for from less than 30 min to perhaps more than 6.5 h. No "calling" was observed until 3 h 15 min after the onset of photophase but thereafter occurred throughout photophase but more often during the second half than the first.

Seven females were seen to walk with the ovipositor fully extended; four of these allowed the ovipositor to touch the glass, and appeared to drag it along the glass for 1-5 mm leaving behind a colourless liquid.

All 15 females also intermittently rested or walked with the ovipositor only partly protruded to less than half the fully extended length (as in Fig. II, 1); 72% of observations of partial extension were proceded and followed by other behaviour, 20% of bouts extended to 2 consecutive occasions, 17% to 3-6 and 1% to 9.

On the day of death, eight females were seen to push the extended ovipositor repeatedly either into the sand or the cotton wool plug, as if attempting to oviposit, an activity that resulted in damage to the ovipositor preventing its retraction.

Of the 13 virgin females paired with a male never seen to "call" presumably they had mated early with the accompanying male. Six of the eight that "called" did so on most days and their behaviour was similar to that of isolated virgin females (Fig. I, b); it was assumed that they had not mated with their accompanying male. The other two called on the first day only but not thereafter, presumably because they had also mated. Mating between a pair was also observed. While one female was "calling" in a stationary position, the male flew to within c. 10 mm of her. He then waved his antennae alternately up and down and raised his forelegs from the ground while rapidly fanning his wings for a few seconds (as in Fig. XIV, 1). The male walked directly to the female, mounted her, copulated for about 20 s, disengaged and departed. During copulation the female's wings were folded in the resting position while the male's were partially raised. The female rectracted her ovipositor partially within a few seconds of connecting with the male and held it ventrally during the copulation. After disengagement the female retracted her ovipositor fully and was not seen to "call" again during her lifetime. This sequence of mating behaviour was subsequently observed in serveral other pairings, amongst midge in culture. Copulation usually lasted 5-20 s but occasionally male and female appeared to have difficulty disengaging and remained connected in the "tail to tail" position for longer, sometimes with the female walking along dragging the male behind her.

The general patterns of behaviour of isolated males were similar to those of males paired with a female (Fig. I: c, d). Much time was spent resting. Flight was observed only from 3 h 15 min to 6 h 45 min after the onset of photophase and walking was observed more during the first half of the observation period than the second.

These observations support the view that virgin female *D. brassicae* produce a sex pheromone from extended ovipositor which attracts males for mating and that females mate only once after which ovipositor extension and sex pheromone relea-

se cease. Williams & Martin (1986) found that in an olfactometer male *D. brassicae* were attracted to hexane washes of ovipositors, but not to washes of the bodies of females without ovipositors, and that in laboratory cages virgin females extended their ovipositors and waved their tips in a circular motion. Mating of a "calling" female by a male has now been described. Further tests, in which 5 virgin females were killed with carbon dioxide on the second day following emergence and their ovipositor extended removed and then presented on filter paper in a Petri dish to a virgin male, elicited an "excited" wing fanning response from the male similar to that observed in response to the "calling" female (Fig. XIV, 2). Premating calling behaviour by the females has also been reported in two other Cecidomyiid midges, *Mayetiola destructor* (SAY) (McKay & Hatchett, 1984) and *Contarinia oregonensis* Foote (Miller & Borden, 1984). Sylvén (1949) observed copulation in *D. brassicae* but reported that it began without ceremony by the male, lasted 30-45 sec and that one male copulated with four females in 100 min.

Ovipositor gross anatomy

The ovipositor of *D. brassicae* is of the Kieffer's (1900) "à pochette" type, i.e., a telescopic one consisting of the last three abdominal segments (8, 9, 10, Figs. II, III: 1). The 8th uromere is much smaller than the preceding segment and made of two distinct portions: an anterior, bearing two characteristic dorsal longitudinal apodemes as long as itself, and a posterior, narrower tubular portion of similar length to the anterior, entirely membranous and caudally terminating with an irregular row of tactile sensilla (TS, Fig. III, 2). The 9th uromere is notably longer than the 8th within which it slides during extension/retraction but always (even in resting position) leaving its caudal end projecting (Fig. II, 1). It is connected by a sleeve-like membrane, i.e. the 8th-9th intersegmental membrane (IM, Figs. II, III, etc.). The 10th uromere consists, as is normal in this taxonomic group, of two membranous lamellae: the superior (tenth urotergum also interpreted as cerci) and the inferior (tenth urosternum).

D. brassicae ovipositor anatomy is very similar to that of *Allocontarinia sorghicola* (Coq.) (Solinas & Isidoro, 1991), including the observation that the epidermis of the 8th-9th intersegmental membrane represents the pheromone gland.

Gland histology, fine structure and physiology

In newly emerged virgin females, the 8th-9th intersegmental membrane epidermis consists of a single layer of cylindrical-cuboidal cells, quite varied in shape and size (PG, Figs. IV, V), resting on a well-developed, rather thick basement membrane (BM, Figs. VII, VIII, IX). These cells have a basal plasma membrane with almost no infoldings (Figs. VI, IX,), while the apical plasma membrane is moderately convoluted (Figs. VII, VIII). The lateral cell boundaries, as normal in epidermal tissue, constantly display septate junctions (SJ, Figs. VII, IX) on the apical portion. These cells possess a large nucleus (N, Fig. VII) with features typical of intense activity, and cytoplasm containing: extensive smooth endoplasmic

reticulum (SE, Figs.VII, VIII, IX); a moderate amount of granular reticulum confined around the nucleus (Fig. VII); abudant ribosomes (R, Figs. VII-IX) isolated or in groups randomly distributed throughout the cell; well-developed mitochomdria (M, Fig. VIII) frequently displaying whorled cristae; obvious Golgi apparatus (G, Fig. IX, 2,3); numerous electronlucid and electrondense secretion vesicles (V, Figs. VII-IX). The cuticle (CU, Figs. VII, IX) overlying the cells in question does not show any obvious porosity, and consists of a thin epicuticle and a relatively thick procuticle that appears quite homogenous and electrondense so that it is usually difficult to distinguish from the epicuticle.

At low magnification, the outer surface of the 8th-9th intersegmental membrane (Figs. II, 2; III: 1, 2) looks quite smooth, but at higher magnification (Fig. III, 3) it can be seen to consist of a continuos series of alternate irregular minute protuberances (P) and irregular grooves (GR), the former bearing some short microtrichia (MI).

Features such as extensive smooth endoplasmic reticulum and numerous and modified mitochondria are typical of pheromone producing cells (Solinas & Isidoro, 1991, and references therein).

Furthermore, the observed ultrastructure of the cuticular outer surface may form a special device for storing the pheromone(s) by retaining it within the grooves when they are closed in the folded (ovipositor retracted) intersegmental membrane. Also, the said cuticular features may regulate the pheromone(s) evaporation when the said membrane is gradually everted, during ovipositor extension, by the calling female midge.

In 24 h old virgin females, the epidermis of the 8th-9th intersegmental membrane, i.e. the pheromone gland, has the same ultrastructural features observed in the newly emerged virgin females (compare Figs. IV-IX with Figs. X-XI).

In 25 h old mated females, fixed 24 h after copulation, the gland cells show an obvious atrophy (compare Figs. XII-XIII with Figs. X-XI) as a consequence of copulation, thus confirming that it is the sex pheromone gland.

Conclusions

The aims of the reported work were to investigate the behaviour associated with mating in *D. brassicae* and to identify by anatomo-physiological means the gland that produces the sex pheromone(s) in this species. From the above results and discussion, the following conclusions may be drawn:

1. *D. brassicae* virgin females attract conspecific males by means of sex pheromone(s).

2. Virgin females release the sex pheromone during "calling" behaviour, i.e., repeated ovipositor extension-retraction.

3. Virgin females can continue "calling" throughout their lives but once mated, females cease "calling".

4. Excised ovipositors from virgin females attract conspecific males and arouse sexual behaviour in them.

5. The ovipositor, consisting of 8th & 9th &10th uromeres, contains the sex pheromone gland, as the epidermis of the 8th-9th intersegmental membrane appears quite different in virgin than mated females. In the former, the epidermis is relatively hypertrophied and with cells displaying ultrastructural features typical of pheromone-producing gland cells; whereas in the latter, the epidermal cells undergo atrophy following copulation.

ACKNOWLEDGEMENTS

We are very grateful to: Mr. E. Mariucci for arrangement of the figures; Mr. A. Mommi for film processing and photographic printing; Mr. C. Dentini for technical assistance in fixing-embedding specimens; Perugia University C.U.M.E. members for assistance in using Philips T.E.M. 400T and S.E.M. 501B; Mr. L. Bonuomo for careful typing the manuscript.

SUMMARY

The Brassica Pod Midge (*Dasineura brassicae* Winn.) is a serious pest of Brassica crops in Europe when combined with infestation of *Ceutorhynchus assimilis* Payk.; the sex pheromone produced by the female pod midge has potential for control.

The presence of a female sex pheromone in *D. brassicae* has been previously demonstrated and the ovipositor suggested as a site of its production and release. In the present work, observations on the mating behaviour are reported and the female sex pheromone gland is identified.

In the laboratory, virgin females of *D. brassicae* extended their ovipositors ("called") to release sex pheromone(s), intermittently throughout their lives but most intensely on the second day of life. "Calling" females were seen to attract males and copulation followed. "Calling" and attraction of males ceased after mating.

Anatomo-physiological studies of the ovipositor of *D. brassicae* identified the sex pheromone gland in the 8th-9th intersegmental membrane epidermis. This epidermis is hypertrophied with ultrastructure typical of pheromone producing gland cells in the virgin female but atrophies following mating.

KEY WORDS: mating behaviour, sex pheromone, anatomy, ultrastructure, physiology, bioassays.

RIASSUNTO

Comportamento sessuale ed identificazione della ghiandola a feromoni sessuali in *Dasineura brassicae* Winn. (Diptera: Cecidomyiidae)

La Cecidomia delle silique del colza e di altre brassicacee (*Dasineura brassicae* Winn.) è comune in Europa ed arreca gravi danni all'Oleaginosa in concomitanza con infestazioni di *Ceutorhynchus* assimilis Payk. Il controllo della Cecidomia con metodi tradizionali è difficile, mentre un contributo valido, considerando il comportamento degli adulti, potrebbe venire dall'impiego di prodotti semiochimici (feromoni compresi). L'esistenza di feromoni sessuali prodotti dalle femmine e dalle medesime diffusi all'atto del richiamo sessuale, tramite allungamento dell'ovopositore, era già nota. Nel presente lavoro viene identificata la ghiandola a feromoni sessuali su basi anatomiche, ultrastrutturistiche, fisiologiche ed etologiche. Detta ghiandola è costituita dall'epidermide della membrana intersegmentale tra gli uromeri 8° e 9°; la quale epidermide nelle femmine vergini presenta le cellule relativamente ipertrofiche e con caratteristiche ultrastrutturali tipiche delle cellule secernenti feromoni, mentre nelle femmine accoppiate dette cellule vanno rapidamente incontro ad atrofia.

REFERENCES

BROMAND B., 1990. - Diversities of oilseed rape growing within the Western Palaearctic Regional Section. - *Bulletin SROP/WPRS - Proceendings of the OILB Meeting Malmö, Sweden, 21-22 March, 1988,* 13(4): 7-31.

FOSTER S.P., BERGH J.C., ROSE S., HARRIS M.O., 1991a. - Aspects of Pheromone Biosynthesis in the Hessian Fly, Mayetiola destructor (Say). - *J. Insect Physiol.,* 37: 899-906.

FOSTER S.P., HARRIS M.O., MILLAR J.G., 1991b. - Identification of the Sex Pheromone of the Hessian Fly, *Mayetiola destructor (Say).- Naturwissenshaften,* 78: 130-31.

GISIN H., 1960. - Collembolenfauna Europas. - Naturwiss., Museum, Geneve.

KARNOSWKY K.N., 1965. - A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. - *J. Cell Biol., 27:* 137 A-8.

KIEFFER J.J., 1900. - Monographie des Cecidomyides d'Europe et d'Algerie. - *Ann. Soc. Entom. France,* LXIX: 181-472.

MCKAY P.A., HATCHETT J.H., 1984. - Mating behaviour and evidence of female sex pheromone in the Hessian fly, Mayetila destructor (Say) (Diptera: Cecidomyiidae). - *Ann. Entomol. Soc. Amer.,* 77: 616-620.

MILLER G.E., BORDEN J.H., 1984. - Reproductive behaviour of the douglas-fir cone gall midge, *Contarinia oregonensis* (Diptera: Cecidomyiidae). - *Can. Entomol.,* 116: 607-618.

PETTERSSON J., 1976. - Ethology of *Dasineura brassicae* Winn. (Dipt., Cecidomyiidae). I. Laboratory Studies of Olfactory Reactions to Host-Plant. - *Symp. Biol. Hung.,* 16: 203-208.

SOLINAS M., ISIDORO N., 1991. - Identification of the female sex pheromone gland in the Sorghum Midge, *Allocontarinia sorghicola* (Coq.) Solinas (Diptera, Cecidomyiidae). - *Redia,* LXXIV (2): 441-446.

STECHMANN D.J., SCHUETTE F., 1978 - Zur endophytischen Eiablage *von Dasineura brassicae* Winnertz, 1853 (Dipt., Cecidomyiidae). - *Z. ang. Ent.,* 85: 412-424.

SYLVÉN E., 1949 - Skidgallmyggan *Dasineura brassicae* Winn. - *Statens Växtskyddanstalt Mededeling,* 54: 1-120.

SYLVÉN E., 1970 - Field Movement of Radioactively Labelled Adults of *Dasineura Brassicae* Winn. (Dipt., Cecidomyiidae). - *Ent. Scand,* 1: 161-187.

WILLIAMS I.H., MARTIN A.P., 1986 - Evidence for a female sex pheromone in the Brassica Pod Midge *Dasineura brassicae.* Winn. - *Physiol. Entomol,* 11: 353-356.

WILLIAMS I.H., MARTIN A.P., KELM M., 1987a - The phenology of emergence of Brassica Pod Midge (*Dasineura brassicae* Winn.) and infestation of winter oilseed rape (*Brassica napus* L.). - *Journal of Agricultural Science,* Cambrige, 108: 579-589.

WILLIAMS I.H., MARTIN A.P., KELM M., 1987b - The phenology of the emergence of Brassicae Pod Midge (*Dasineura brassicae* Winn.) and its infestation of spring oilseed rape (*Brassica napus* L.). - *Journal of Agricultural Science,* Cambridge, 109: 309-314.

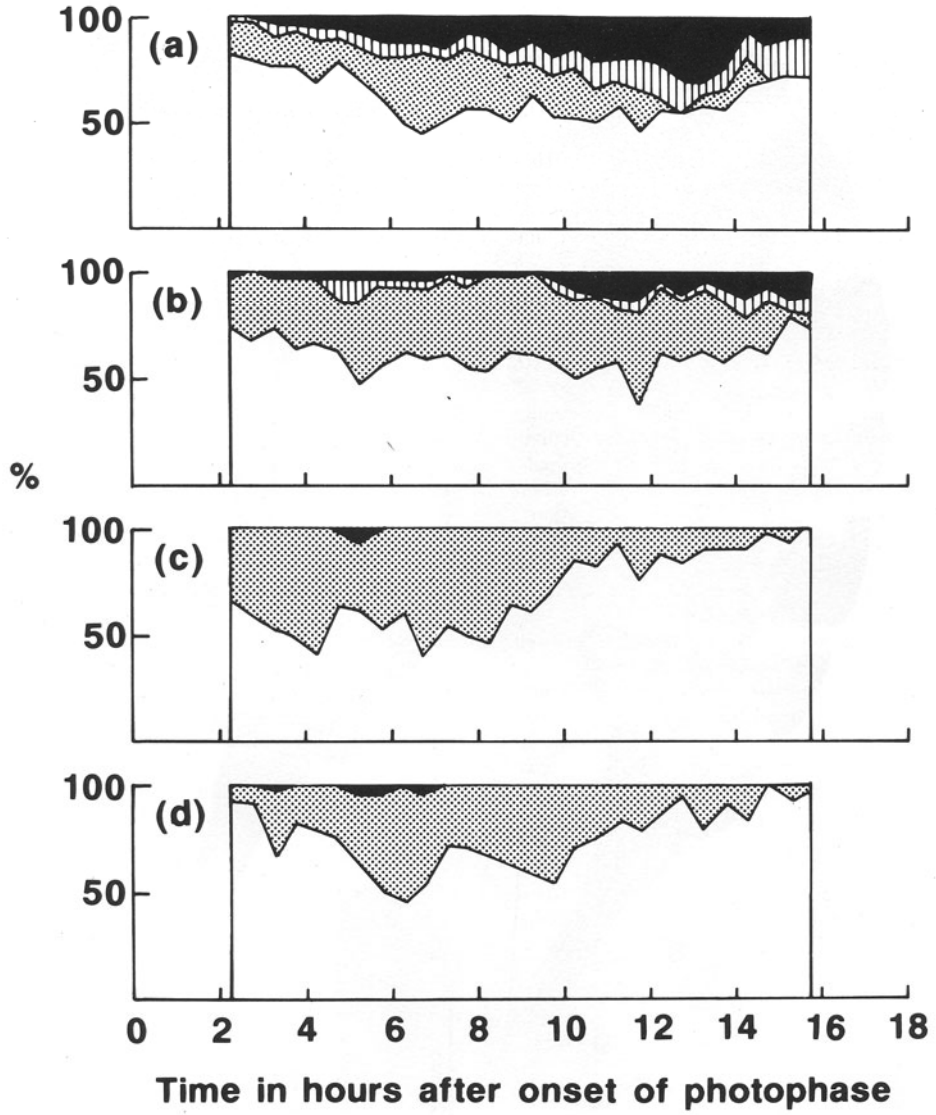

Fig. I - Diurnal periodicity of activity of *Dasineura brassicae* adults kept at 18°C on LD 16: 8 h cycle and observed from 2 h 15 min after the onset of photophase.
(a) Isolated females (n= 15); (b) females paired with a male (n= 13). □, stationary with ovipositor retracted; ⊡, walking with ovipositor retracted; ▥, stationary (or occasionally walking) with ovipositor partially protruted; ■, "calling", i.e., stationary with ovipositor fully extended.
(c) Males paired with a female (n= 13); (d) isolated males (n= 11). □, stationary; ⊡, walking; ▥, flying.

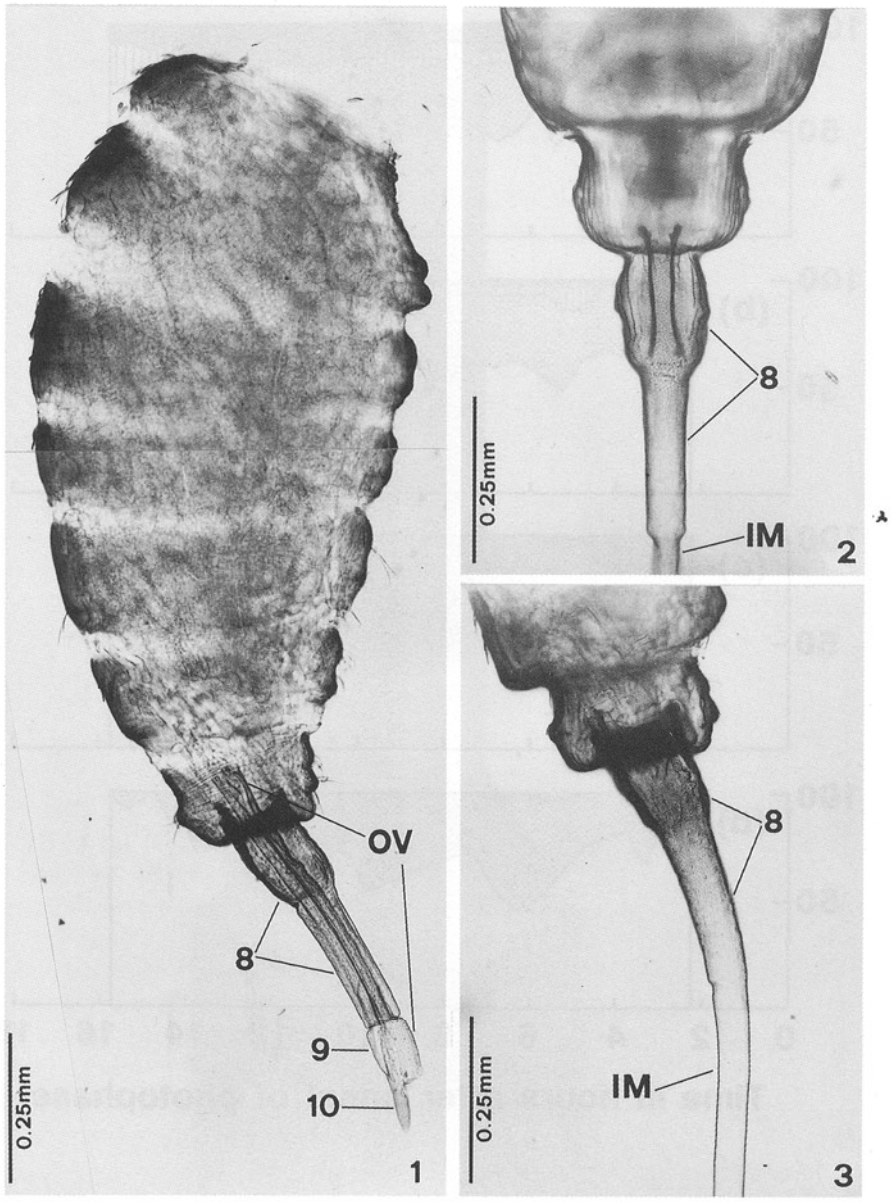

Fig. II - *Dasineura brassicae* Winn. Photomicrographs: 1. female whole abdomen (right side view) with ovipositor (partially) extended; 2. ovipositor (fully-extended) base, dorsal aspect; 3. the same, right side view. (2 and 3 at the same magnification).

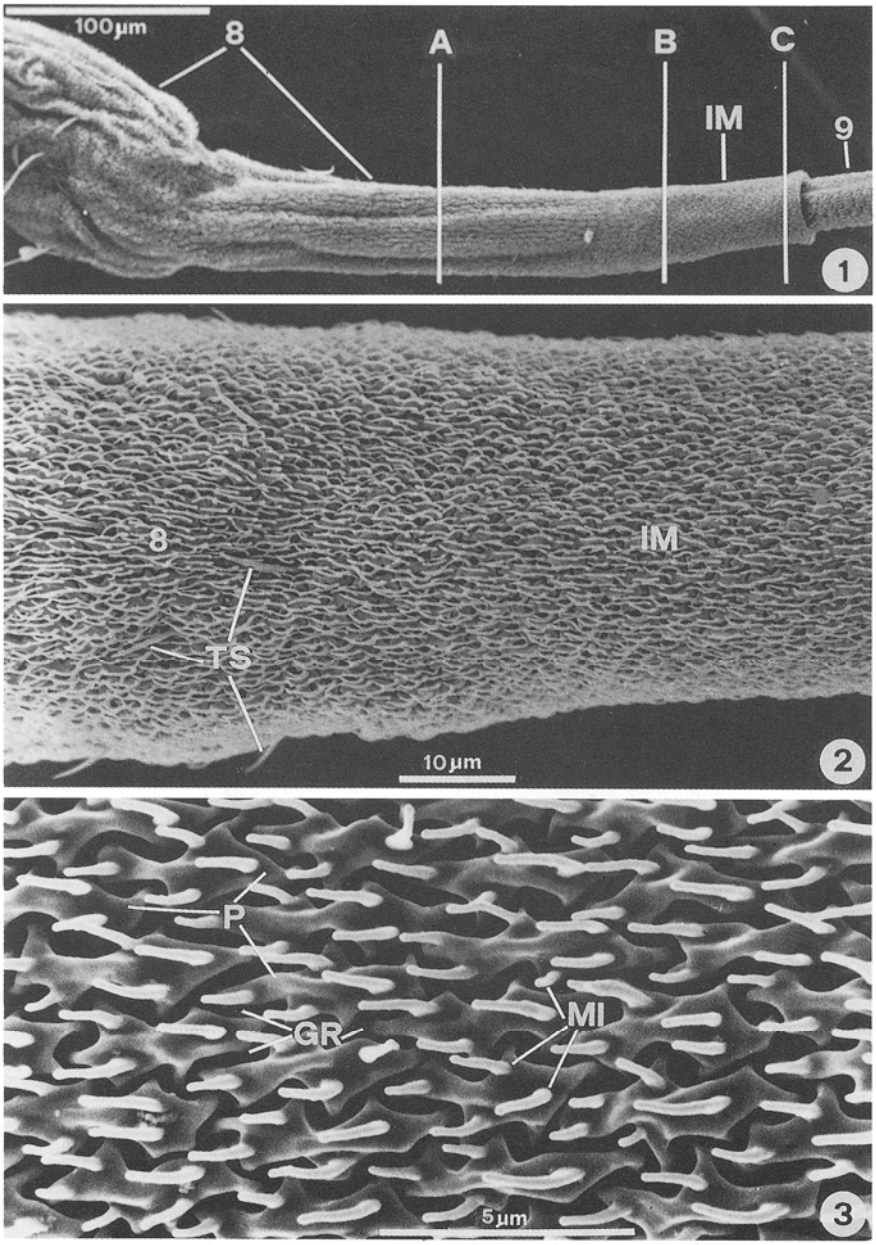

Fig. III - Scanning electronmicrographs showing: 1. ovipositor left side view with eighth uromere fully extended and 8th-9th intersegmental membrane partially extended; 2. detail of the same displaying the eighth uromere posterior end (bearing tactile sensilla, TS) followed by the intersegmental membrane (IM); 3. detail of the outer surface of the membrane.

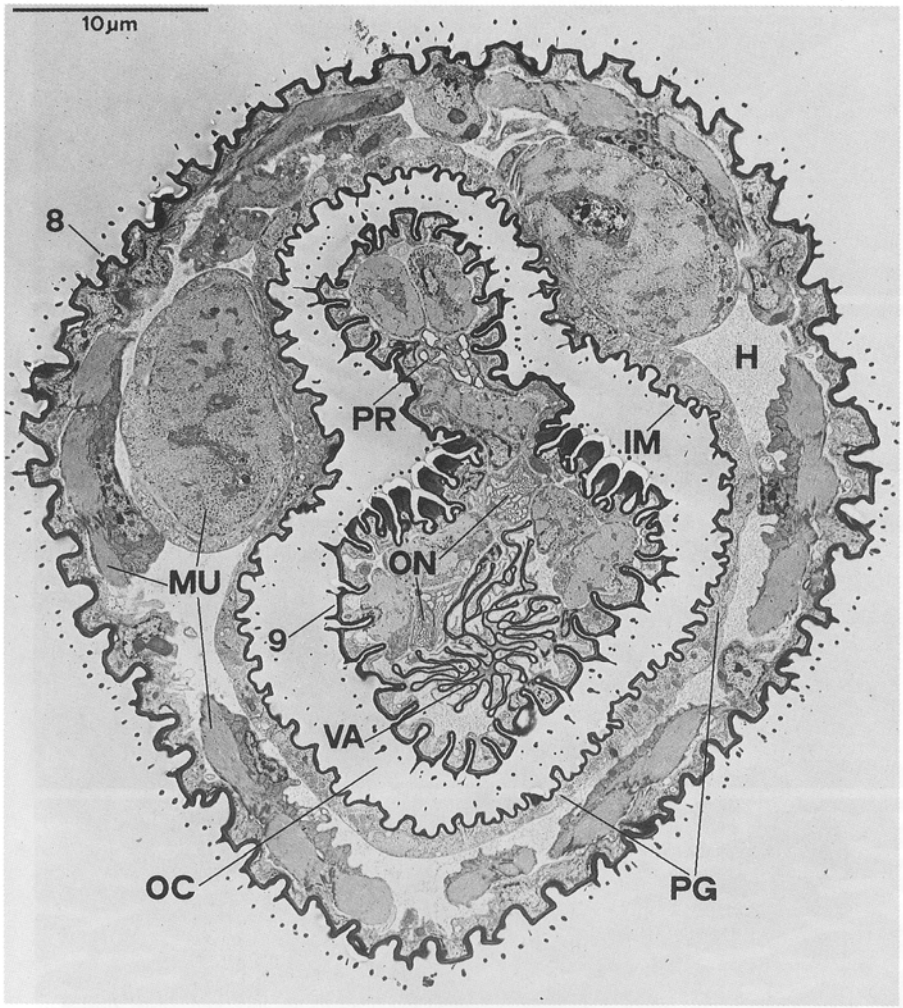

Fig. IV - Newly emerged virgin female, ovipositor (partially extended) cross section through eighth uromere tubular portion (8) at about half length of this (A level, Fig. III, 1), intersegmental membrane (IM) near its posterior end, and ninth uromere (9) near its anterior end. (Dorsal side on the top).

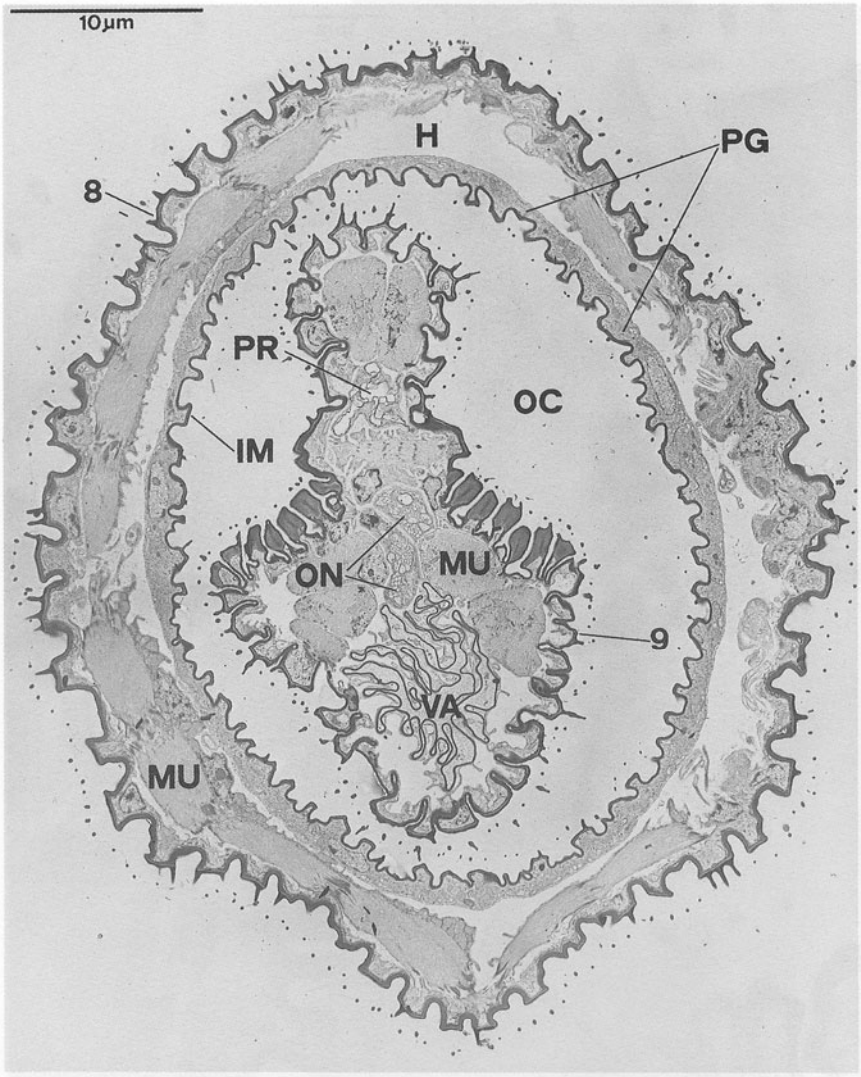

Fig. V - Newly emerged virgin female, ovipositor (partially extended) cross section through eighth uromere (8) posterior end (B level, Fig. III, 1), intersegmental membrane (IM) near its posterior end, and ninth uromere (9).

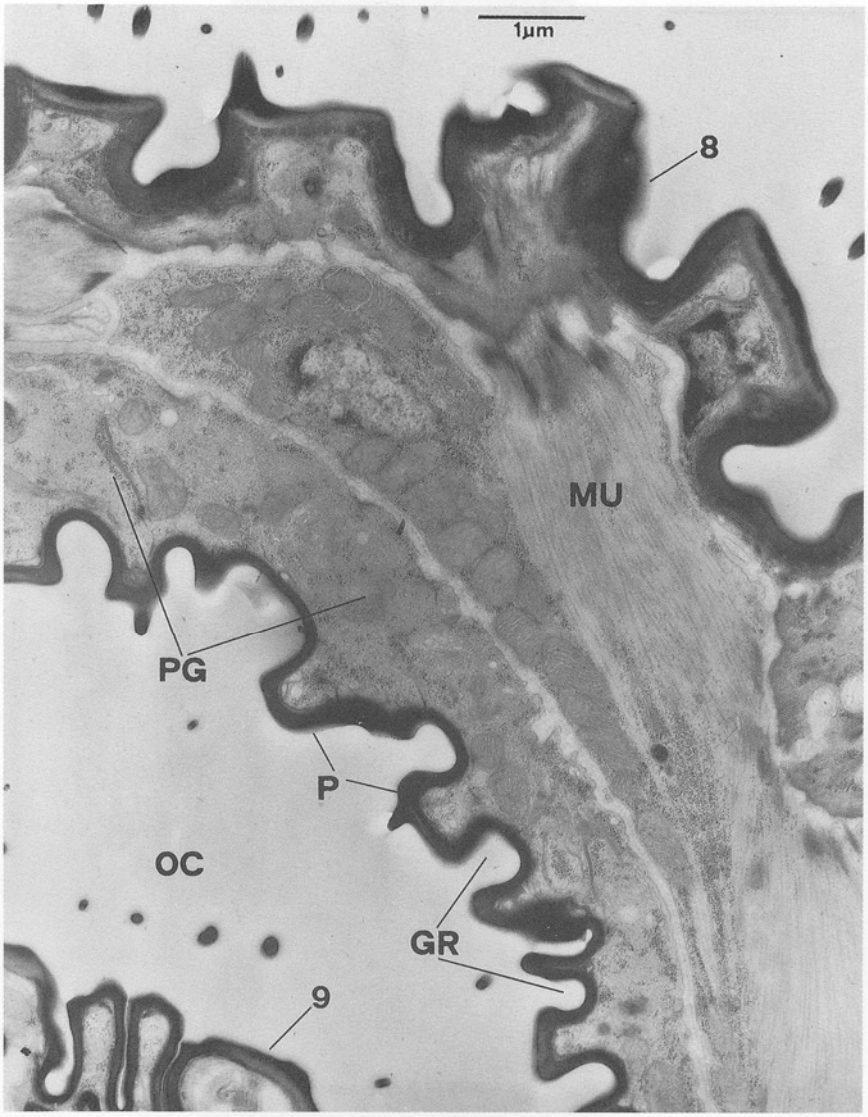

Fig. VI - Newly emerged virgin female, a detail of ovipositor (partially extended) cross section at B level (Fig. III, 1).

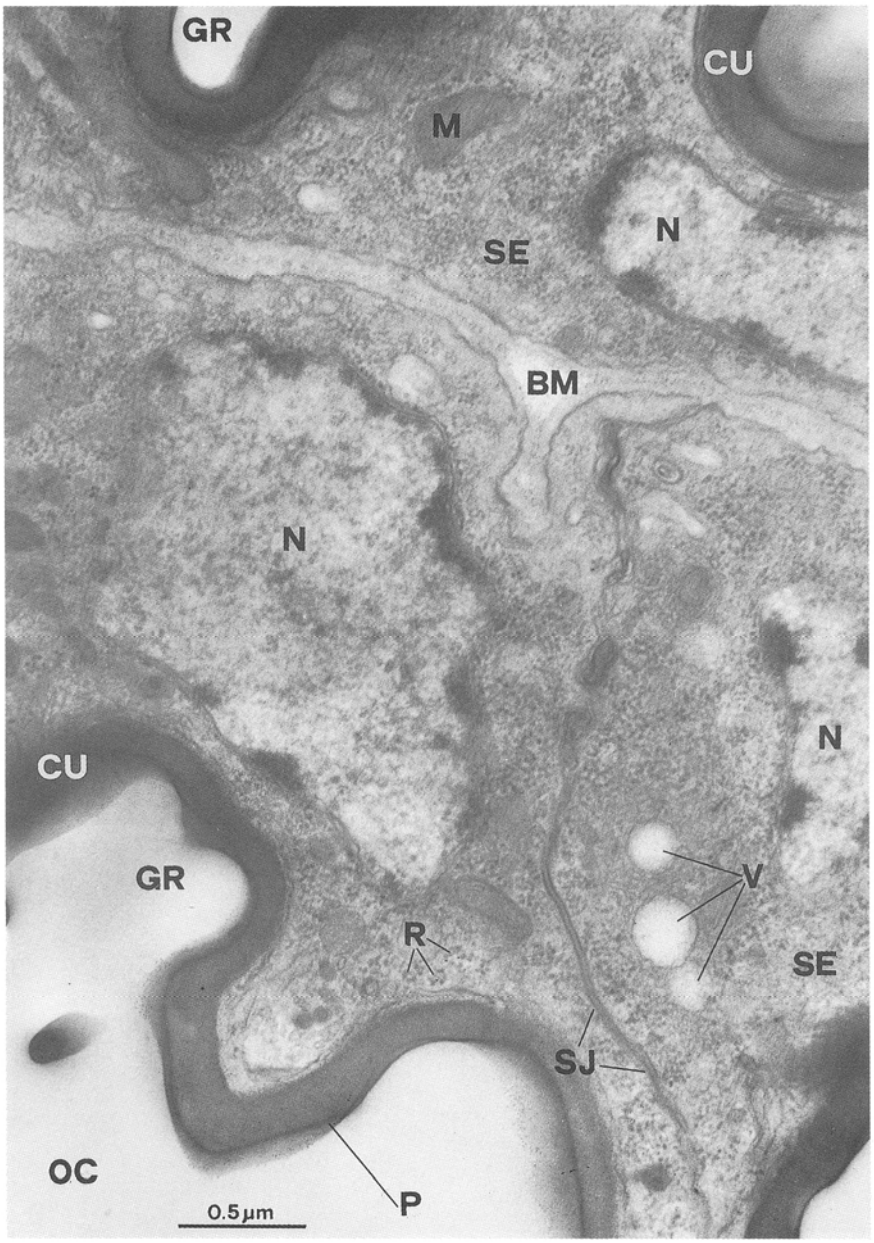

Fig. VII - Newly emerged virgin female, a detail of folded 8th-9th intersegmental membrane cross section through C level (Fig. III, 1) showing pheromone gland cell details.

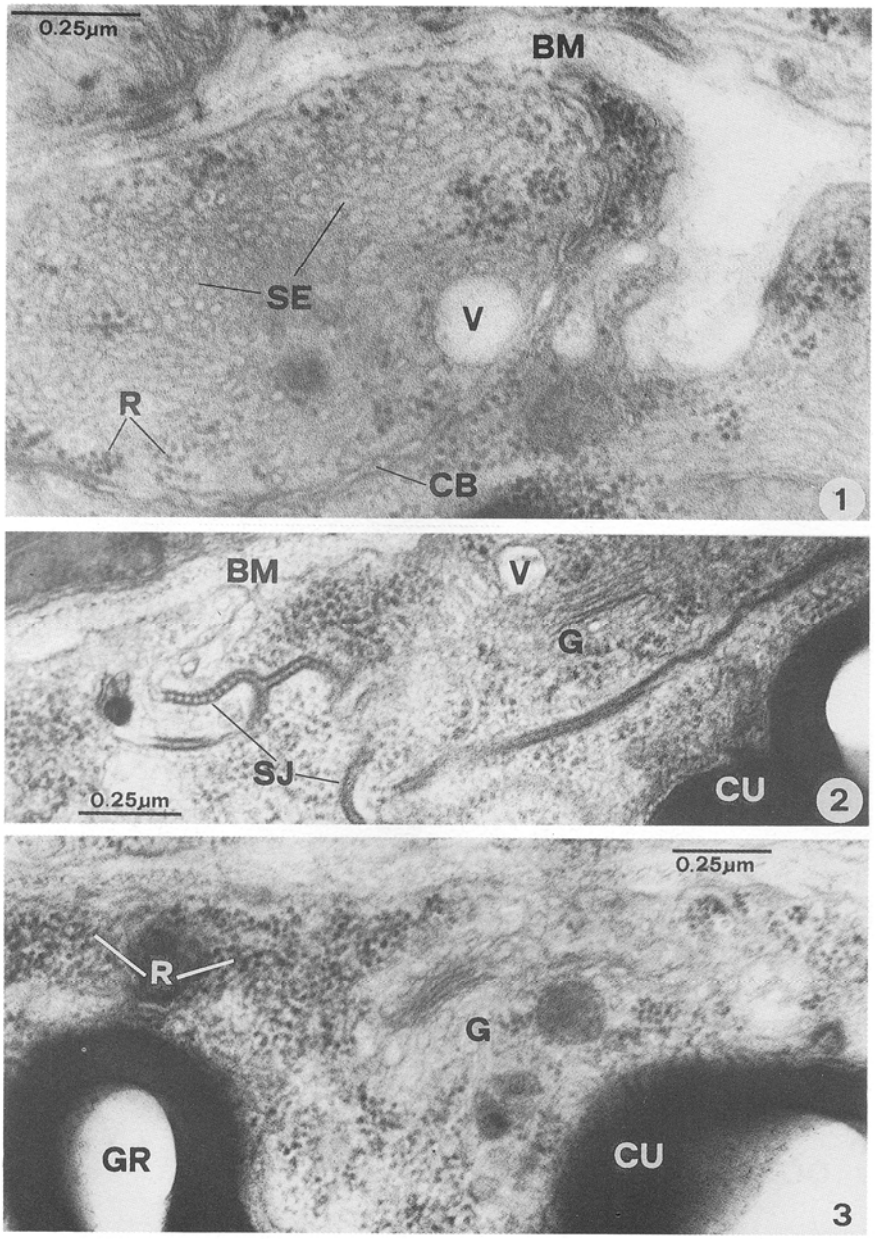

Fig. VIII - Newly emerged virgin female, pheromone gland cell details.

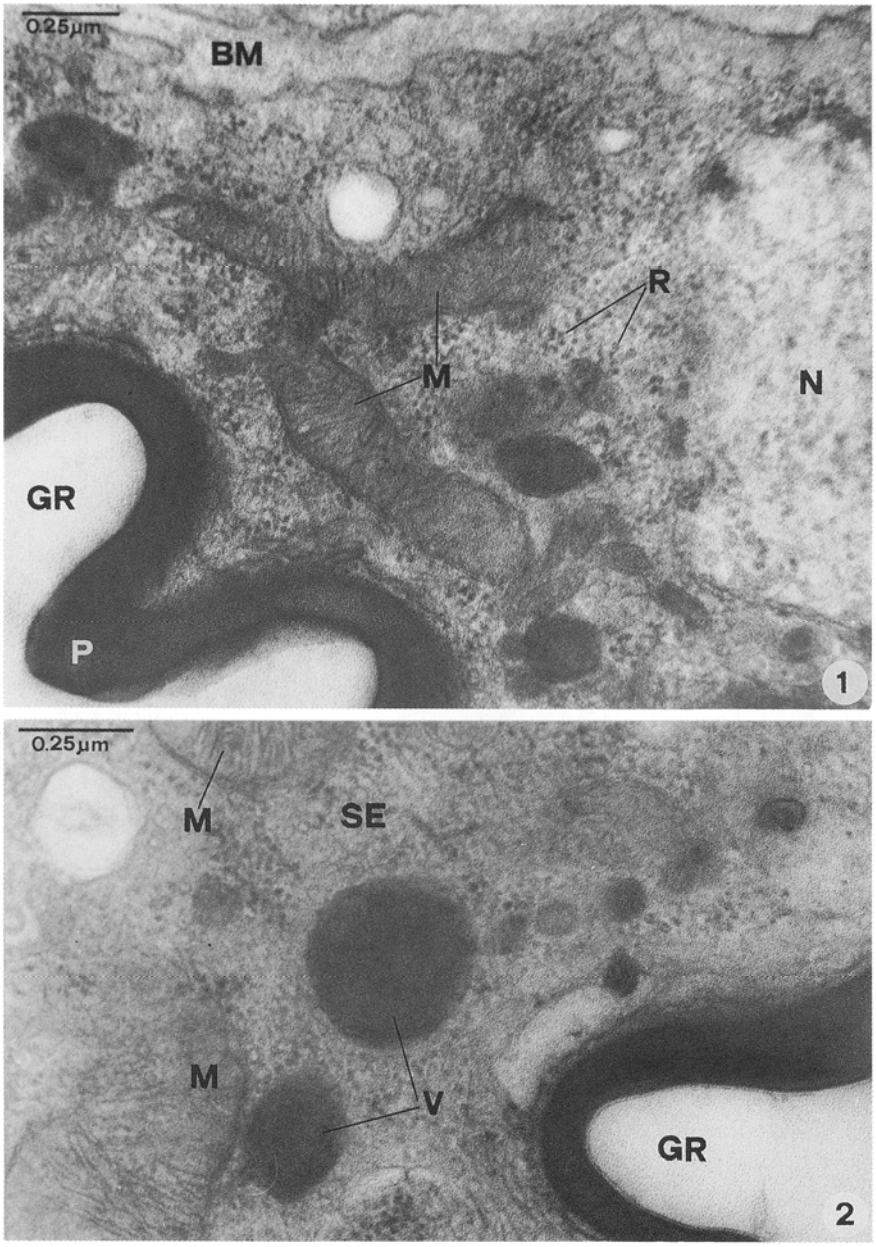

Fig. IX - Newly emerged virgin female, pheromone gland cell details.

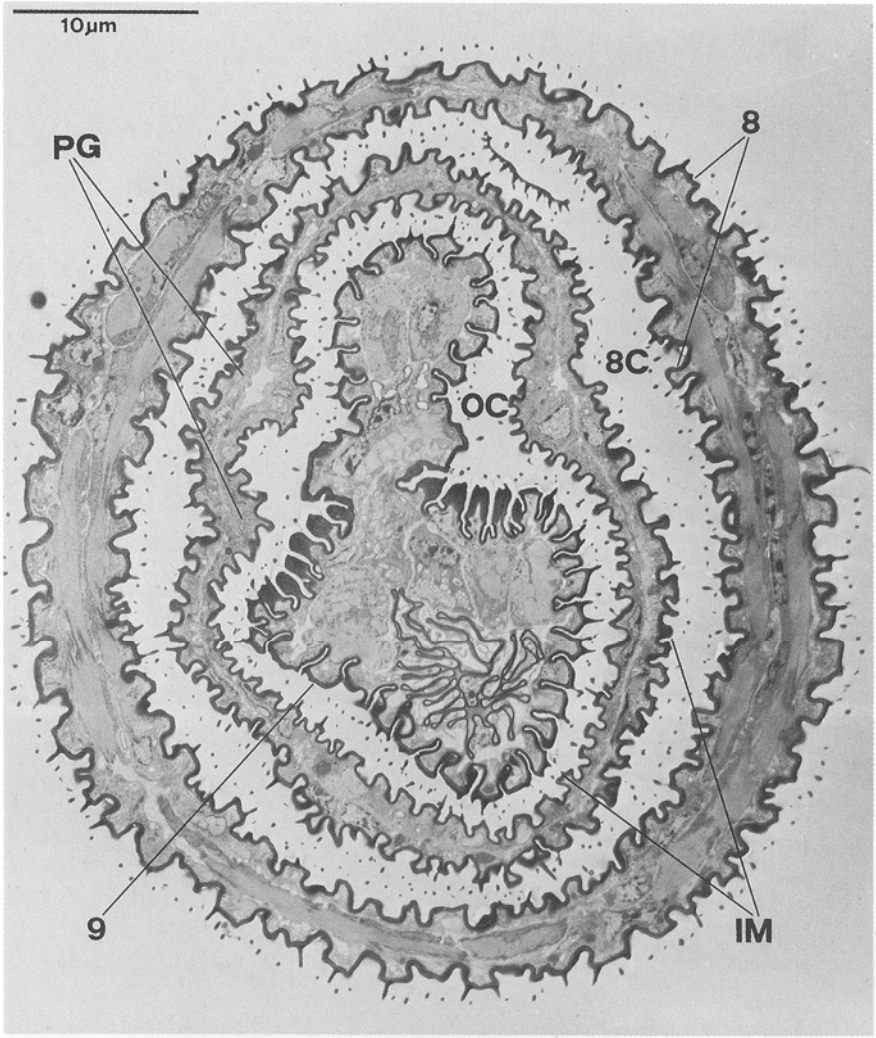

Fig. X - 24 h old virgin female, ovipositor (partially extended) cross section through eighth uromere tubular portion (8, near posterior end a little retracted), folded intersegmental membrane (IM) and ninth uromere (9).

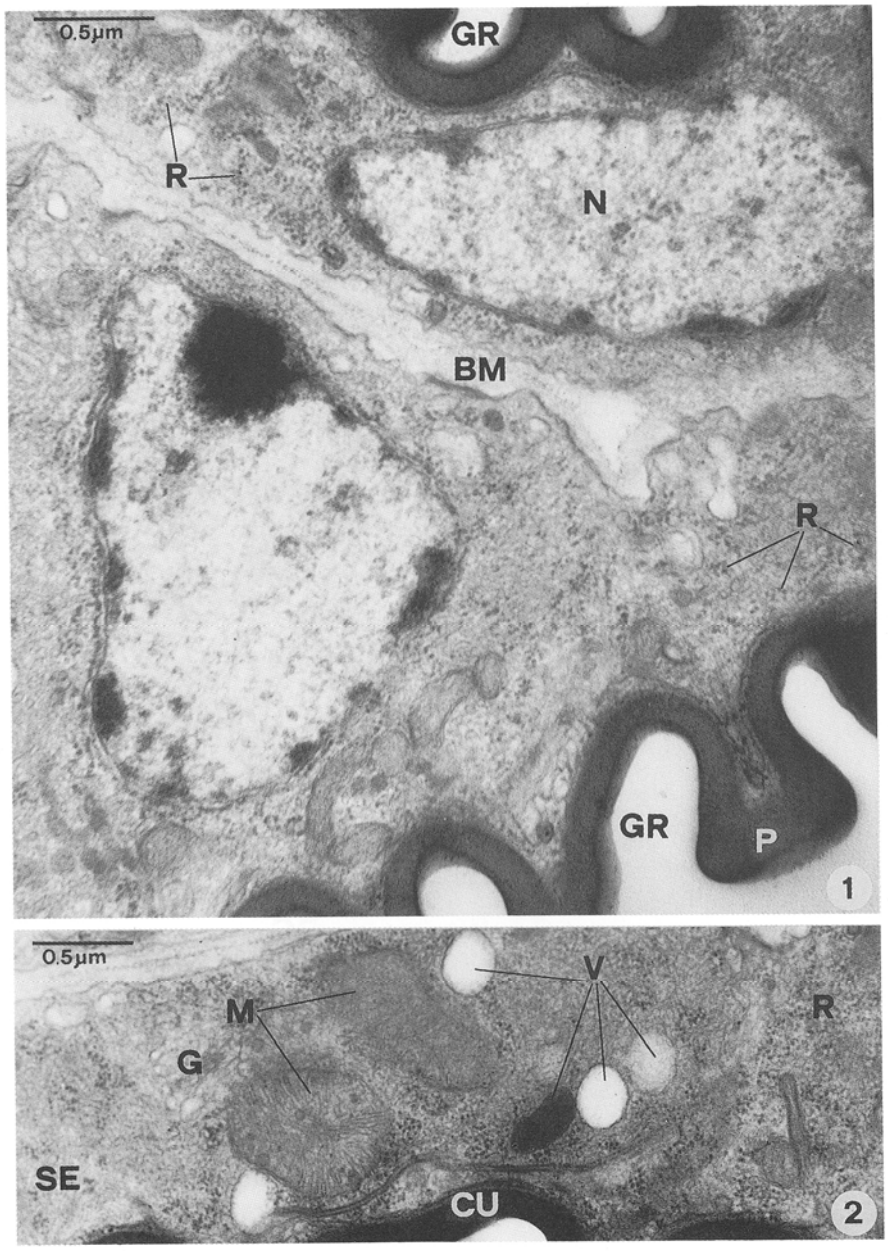

Fig. XI - 24 h old virgin female, pheromone gland cell details.

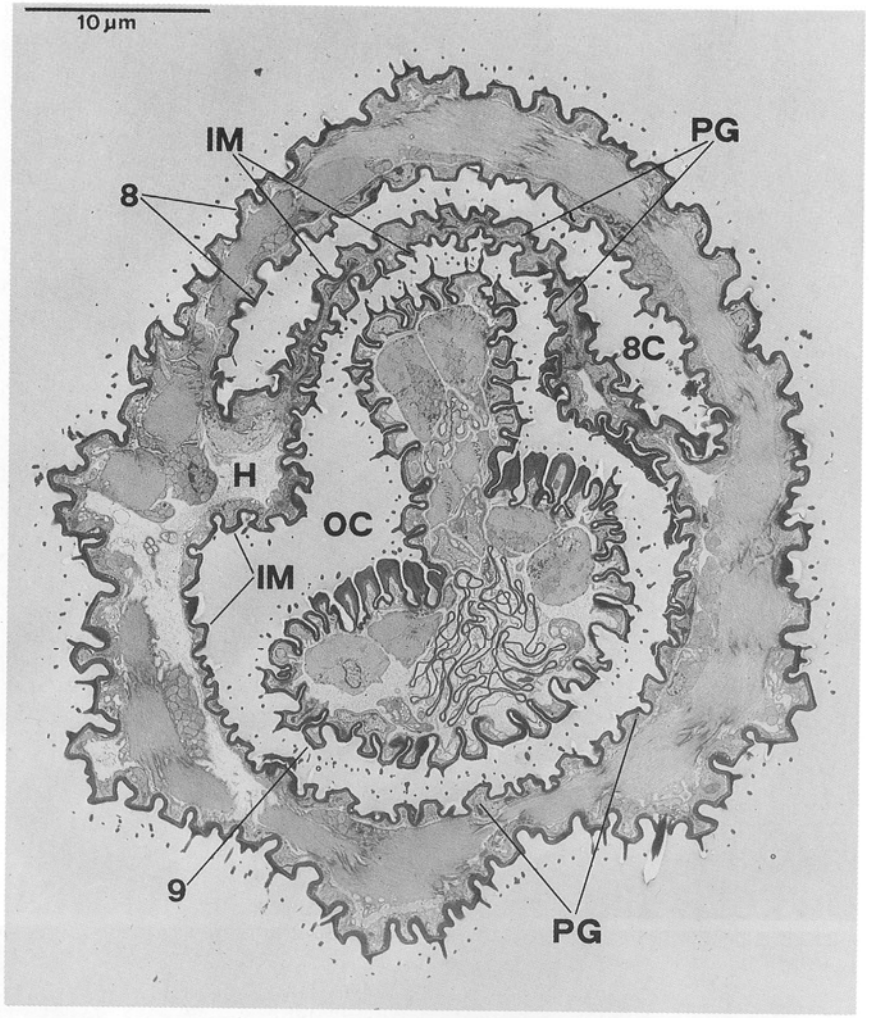

Fig. XII - 25 h old mated female, fixed 24 h after copulation, ovipositor (partially extended) cross section through eighth uromere tubular portion (8, near posterior end dorsally a little retracted), retracted intersegmental membrane (IM, dorsally folded on itself) and ninth uromere (9).

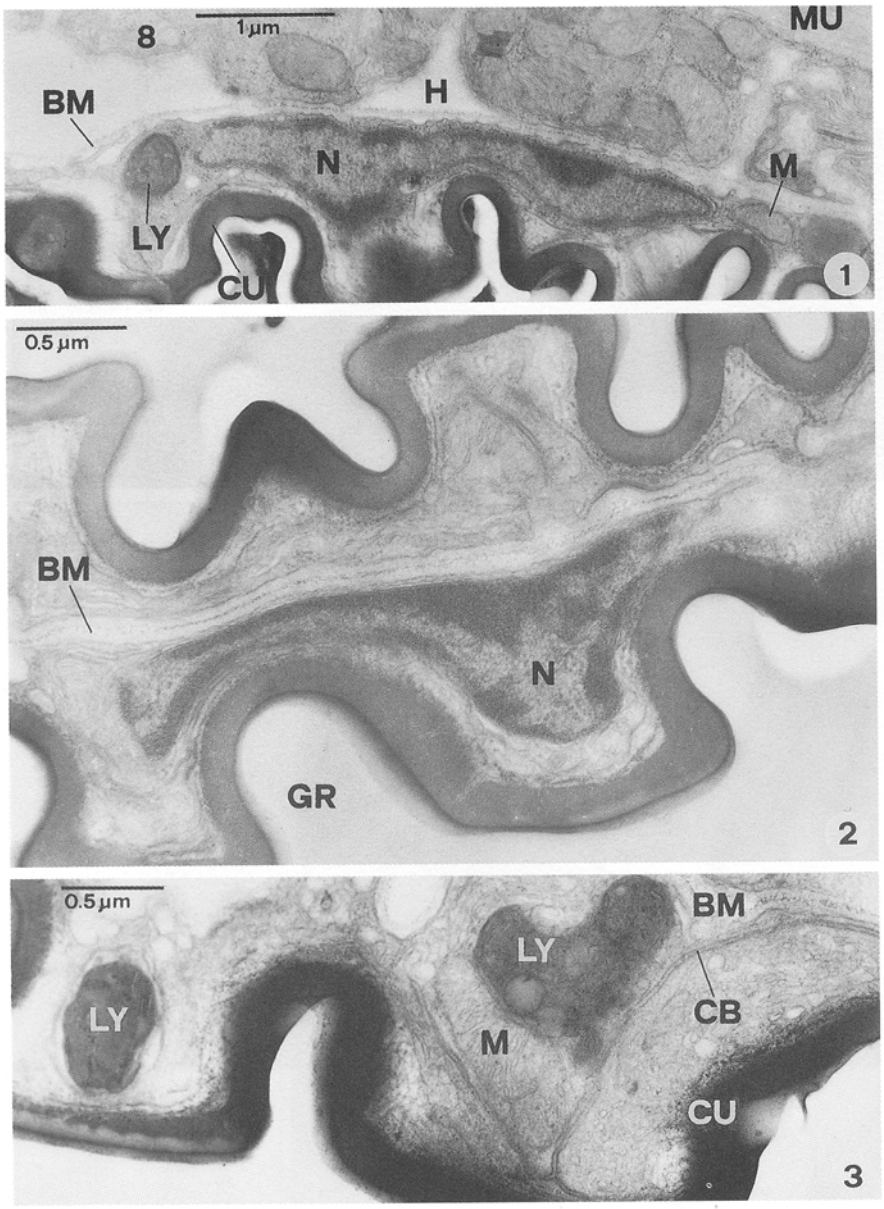

Fig. XIII - 25 h old mated female, fixed 24 h after copulation, pheromone gland cell details.

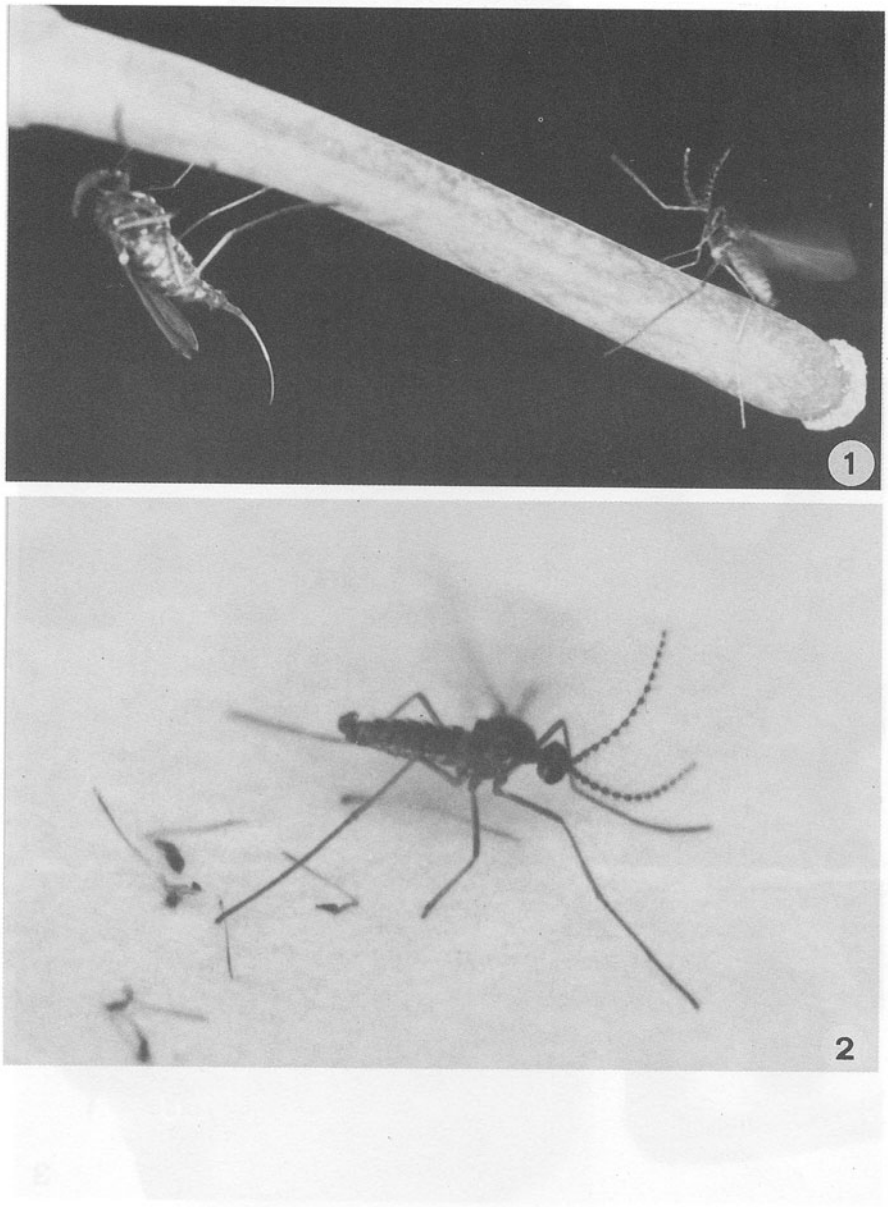

Fig. XIV - 1. *Dasineura brassicae* Winn. virgin female hanging on the tip of a *Brassica napus* pod and displaying "calling" behaviour, and an obviously excited conspecific male just landed on the same pod; 2. male patently excited over extended ovipositors freshly excised from virgin females.