SEX PHEROMONE GLAND OF THE NAVEL ORANGEWORM, *AMYELOIS TRANSITELLA* (LEPIDOPTERA: PYRALIDAE) LOCATION, BIOASSAY AND *IN VITRO* MAINTENANCE

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ABSTRACT

Anatomical studies of the sex pheromone gland of the female navel orangeworm, *Amyelois transitella* (Walker), revealed the gland to be a broad, chevron-shaped structure located on the ventrolateral surface of the intersegmental membrane between abdominal segments VIII and IX. Support for this finding was obtained by bioassay and gas chromatographic analyses of extracts that were prepared from various portions of the terminal abdominal segments of female moths. Histological examination showed that the gland consists of a single layer of specialized columnar epidermal cells.

Sex pheromone glands were obtained from surface-sterilized abdominal segments of 2 to 3-day-old virgin females and subsequently cultured for up to 7 days in either chemically defined or modified Grace's medium. Bioassays of extracted medium in which sex pheromone glands had been maintained indicated that more pheromone was recovered from modified Grace's medium than from the chemically defined growth substrate.

RESUMEN

Estudios anatómicos de la glándula de feromona sexual de la *Amyelois transitella* (Walker), reveló que la glándula era una estructura ancha en forma de cheurón localizada en la superficie ventro-lateral de la membrana intersegmental entre los segmentos abdominales VIII y IX. Este descubrimiento es apoyado por bio-ensayo y analysis de gas cromatográfico de extractos que fueron preparados de varias porciones de los segmentos terminales del abdomen de polillas hembras. Exámenes histológicos demostraron que la glándula consiste de una sola capa de clulas especializadas de epidermis columnar.

Glándulas de feromonas sexuales fueron obtenidas de la superficie esterilizada de segmentos abdominales de hembras vírgenes de 2 a 3 días de edad, y subsecuentemente cultivadas hasta 7 días en un medio químicamente definido o en el medio Grace modificado. Bio-ensayos del medio extraido en el cual las glándulas de feromonas sexuales han sido mantenidas, indicaron que más feromonas fueron recuperadas del medio Grace modificado, que de sustratos de medios químicamente definidos.

Although recent studies have revealed much about the biosynthesis of lepidopteran sex pheromones, more detailed and comparative studies of biosynthetic pathways would be facilitated greatly if the pheromone-producing tissue could be maintained in tissue culture (Roelofs & Bjostad 1984). Also, in spite of extensive studies on the chemistry of lepidopteran sex pheromones only limited information is available on the control mechanisms involved in the development and activity (production and/or release of pheromone) of sex pheromone glands, and most of these studies have been conducted *in vivo* (Cardé & Webster 1981, Sasaki et al. 1983, Raina & Klun 1984, Webster & Cardé 1984). Again, the understanding of the control mechanisms would be enhanced

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greatly if the sex pheromone gland could be maintained under defined conditions in tissue culture. Ultimately, successful tissue culture of pheromone glands could result in the establishment of *in vitro* pheromone production systems that would facilitate isolation and identification of the biologically active compounds.

Two studies of the *in vitro* culture of sex pheromone glands of pyralid moths have been reported. White et al. (1972) found that 50 or 100 μg/ml of juvenile hormone (JH) were essential for maintaining the integrity of the gland of female sugarcane borers, *Diatraea saccharalis* (F.), in tissue culture. In contrast, Srinivasan et al. (1979) reported that the gland of the female Indian meal moth, *Plodia interpunctella* (Hübner), was successfully maintained *in vitro* without the addition of exogenous JH. This apparent contradiction regarding the JH requirement prompted the present investigation of the *in vitro* maintenance of the gland of another pyralid, the navel orangeworm, *Amyelois transitella* (Walker). *A. transitella*, a major pest of almonds in California, was selected because some aspects of the biology and chemistry of its sex pheromone are known (Srinivasan 1969, Coffelt et al. 1979a,b). Also, Oberlander (1976) had reported the successful *in vitro* maintenance of imaginal discs and fat bodies of *A. transitella*.

This paper reports the requirements for *in vitro* maintenance of the sex pheromone gland of this species and also presents behavioral data that suggests that sex pheromone can be extracted from cultured glands.

Materials and Methods

Moths used in this study were reared as described by Coffelt et al. (1979a) at 27±1°C and ca. 60% RH under a 14:10 (L:D) photoperiod. Photophase and scotophase light intensities were >250 and ca. 0.3 lux, respectively. The larval diet was wheat bran, honey, glycerol (u.s.p.) and tap water (24:2:2:1, vol:vol) that had been autoclaved 15 min at 120°C. Brewer's yeast powder (5% by wt.) was added after autoclaving.

Bioassays and chemical analyses were conducted using the procedures of Coffelt et al. (1979a,b) to confirm the location of the glands. Extracts for these studies were prepared in the following manner: the terminal seqments of the abdomen of 2 to 3-day-old sexually mature females were extruded by gently squeezing the base of the abdomen, and then cut into 3 sections over a small piece of dry ice under a dissecting microscope at 10X magnification. The 1st section was the tip of the 9th segment (fraction A), the 2nd section consisted mainly of the intersegmental region between segments VIII and IX (fraction B), and the 3rd section consisted of the remaining portions of the 8th and 7th segments (fraction C). Hexane extracts were prepared from each of the 3 sections (fractions A, B and C), and the volume of the solvent was adjusted to yield 1 X 10^{-2} female equivalents (FE) per 10 μl. The solutions were bioassayed using activation as a response criterion (Coffelt et al. 1979a). Applicators treated only with hexane served as controls. Gas chromatographic analyses were conducted on a 1.8-m x 2-mm (ID) glass column packed with 3% OV-1 on 100/200 mesh Gas Chrom-Q; and temperature programmed from 100°C at injection to 220°C at 6°C per min.

The procedure for tissue culture of *A. transitella* glands was similar to that of Srinivasan et al. (1979) for *P. interpunctella*. Material for tissue culture was obtained from surface-sterilized (15-20 min immersion of whole insect in 0.1% $HgCl_2$ solution containing 0.1% Triton-X-100) 2 to 3-day-old unmated females. The abdominal tips that contained the glands were rinsed several times in culture medium before being placed in culture. Groups of 10 glands were cultured in sterile glass Petri dishes each containing 1 ml of the culture medium. Cultures were maintained in an incubator at 25°C and 65% RH. Growth substrates investigated were chemically defined Grace's medium, a modified Grace's medium containing whole egg ultrafiltrate, fetal calf serum and bovine

serum albumin fraction V (Yunker et al. 1967), and Ringer's solution (Carolina Biological Co.).

At periodic intervals (1, 3 and 6 days), 2 to 4 glands were removed from these cultures or from virgin females of similar age, fixed in alcoholic Bouin's solution, washed several times with deionized water, and stored in 70% ethanol for histological studies. These studies were conducted using the standard techniques for paraffin-embedded tissue. The abdomens of 2 to 3-day-old virgin females were fixed in alcoholic Bouin's solution for 12 h. The tissue was first embedded in 3% agar before being embedded in paraffin. Sections were cut at 6 μ and stained with hematoxylin and counterstained with eosin.

The glands and medium in which they were cultured were separately extracted after 144 h (6 days) of incubation. Unused medium was also extracted to serve as a control. All extracts were bioassayed in serial dilution to establish the relative quantities of pheromone present in each preparation.

Results and Discussion

The sex pheromone gland of female *A. transitella* is a broad, chevron-shaped structure that is located on the ventrolateral surface of the intersegmental membrane between abdominal scleromata VIII and IX. Figure 1A shows a longitudinal section of the gland of a 2 to 3-day-old female. It consists of a single layer of specialized columnar epidermal cells; its location and morphology are strikingly similar to that reported by Smithwick & Brady (1977) for the confamilial *P. interpunctella*. Bioassays of the extracts of various abdominal parts (Table 1), revealed that >90% of the pheromone (based on additional bioassays of serial dilutions) (see Coffelt et al. 1979a) was contained in fraction B prepared from the intersegmental region between abdominal segments VIII and IX. The low level of male response observed in fractions A and C was probably due to contamination of the surface of the segment during the process of extrusion of the abdominal tip. Gas chromatographic analysis of the biologically active fraction showed the presence of a single peak with a retention time (11.4 min) coincident with synthetic (*Z*,*Z*)-11,13-hexadecadienal, a previously identified pheromone component of *A. transitella* (Coffelt et al. 1979b). No pheromone was found in analyses of fractions A and C.

Earlier studies by both Dickens (1936) and Srinivasan (1969) reported the gland in

TABLE 1. Comparison of the response of *A. transitella* males to the female sex pheromone extracts prepared from the 3 different sections of 25 abdominal tips.[a]

Treatment	% Male response[d]	±S.E.
Control[b]	3.8 a	2.6
Fraction A[b]	16.3 b	3.7
Fraction B[c]	80.8 c	3.3
Fraction C[b]	16.3 b	4.2

[a]Fraction A—Section of the tip of segment IX; fraction B—intersegmental region between segments VIII and IX; and fraction C—remaining portion of segments VII and VIII.
[b]Based on 12 replications of 10 males/replication.
[c]Based on 18 replications of 10 males/replication.
[d]Values followed by the same lower case letter are not significantly different at the 5% level by Duncan's New Multiple Rang Test.

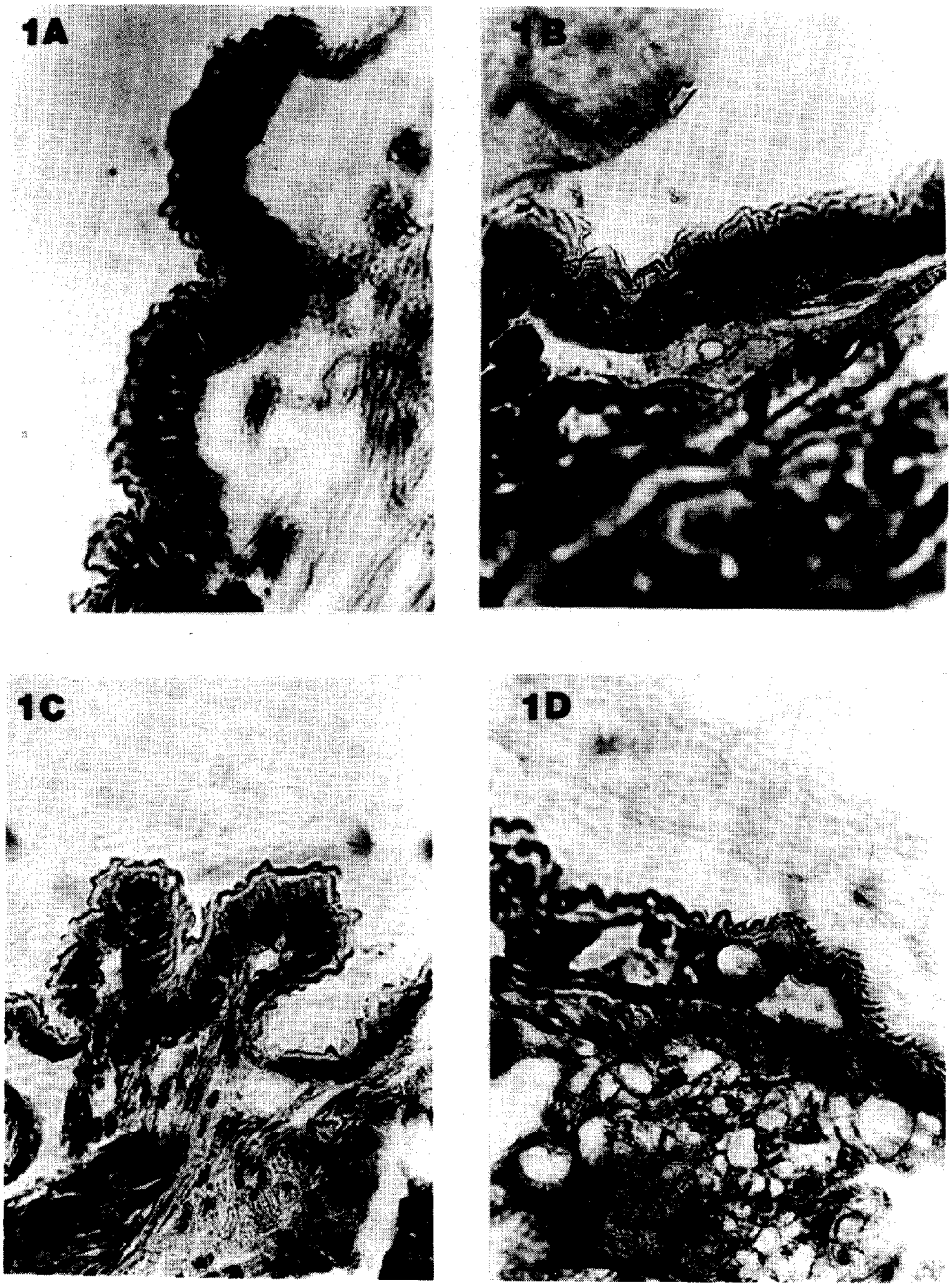

Fig. 1. Sections of 2 to 3-day-old virgin female *A. transitella* glands. (A) Before being placed in culture; (B) after 7 days in chemically defined Grace's medium; (C) after 7 days in modified Grace's medium; and (D) after 7 days in Ringer's solution.

A. transitella females to be a ring of glandular epithelium. However, careful examinations during this study revealed it to be ventrolateral and chevron-shaped. Serial sections of the dorsal region did not show the presence of glandular epithelial cells. Even though *A. transitella* is 2-3X larger than *P. interpunctella*, the gland cells are similar in size to the *P. interpunctella* cells (Smithwick & Brady 1977). We did not determine

TABLE 2. DETECTION OF THE FEMALE SEX PHEROMONE OF *A. TRANSITELLA* BY BIOASSAY FROM TEN GLANDS MAINTAINED IN TISSUE CULTURE FOR 144 H.[1]

Source	% Male response[2]	
	Defined medium	Modified medium
Gland extract	10.0 a	23.3 b
Medium extract	6.7 a	50.0 c

[1]Based on 3 replications of 10 males/replication.
[2]Values followed by the same lower case letter are not significantly different at the 5% level by Duncan's New Multiple Range Test.

the actual surface area occupied by the gland but it appeared slightly larger than that of *P. interpunctella* although not as large as the relative size of the moth would suggest.

Figure 1A shows the gland of a 2-3-day-old virgin female *A. transitella* before it was placed in culture. There was no detectable difference in the histological appearance of the cells of isolated glands maintained in either chemically defined Grace's medium (Fig. 1B), or modified Grace's medium (Fig. 1C). However, the cells in the glands maintained in Ringer's solution had disintegrated (Fig. 1D). Bioassays revealed the presence of sex pheromone only in the extracts prepared from the modified Grace's medium in which the glands had been incubated for 144 h (Table 2). Further analysis of these extracts by as chromatography indicated that quantities of sex pheromone recovered were below the detection limits (<0.1 ng/FE).

Like *P. interpunctella* (Srinivasan et al. 1979), the *A. transitella* glands did not require exogenous JH in the medium as reported by White et al. (1972) for the sugarcane borer and also could be maintained structurally in chemically defined Grace's medium. The materials present in the modified Grace's medium, thus were not essential for gland survival, but were necessary if pheromone was to be recovered from the culture medium.

END NOTES

This research was supported in part by NIH Grants I-F34G1706251 and 5-506RR8110. Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.

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INFLUENCE OF CABBAGE CULTIVAR AND FREQUENCY OF INSECTICIDE APPLICATION ON DAMAGE BY THE CABBAGE LOOPER (LEPIDOPTERA:NOCTUIDAE)

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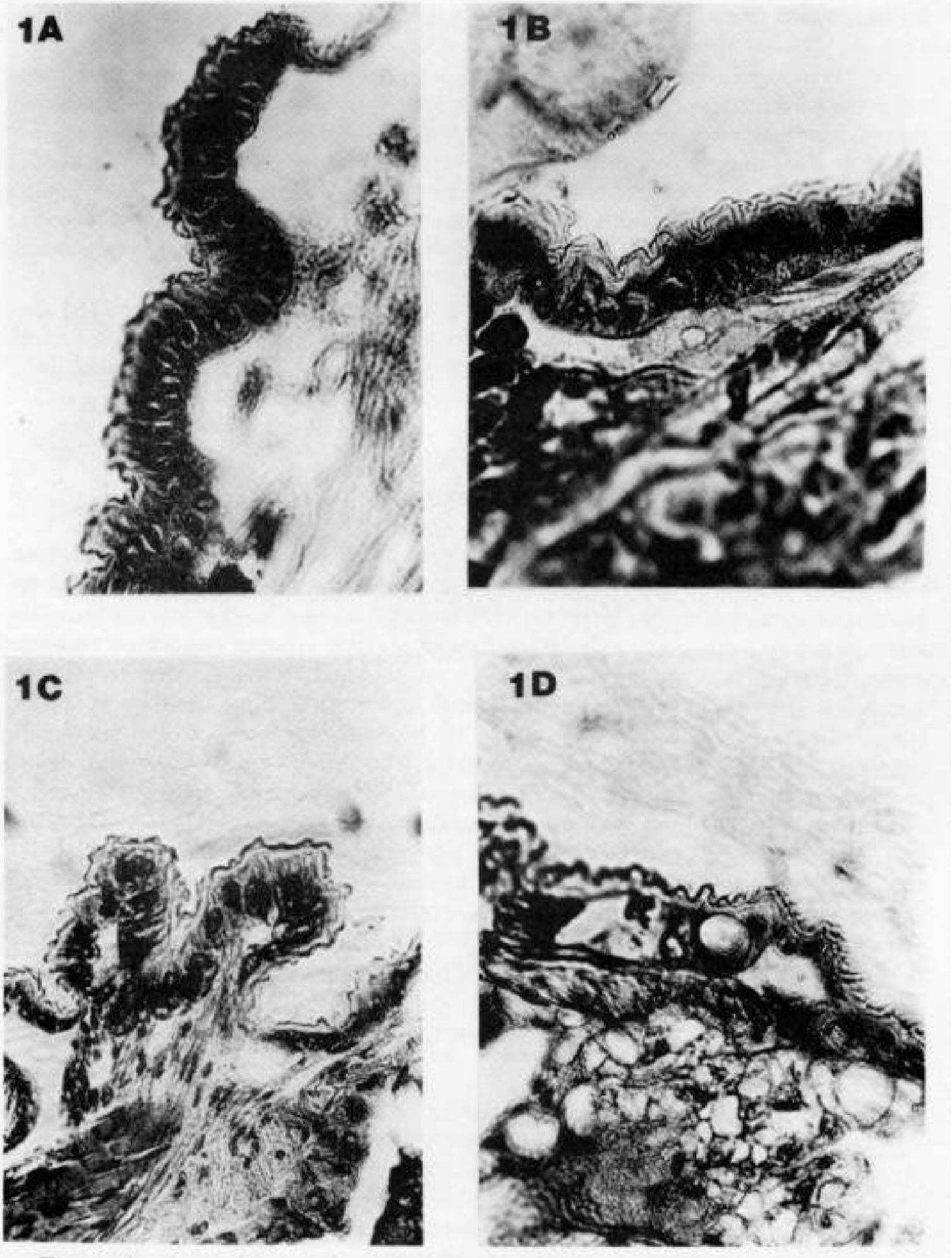
ABSTRACT

Field tests were conducted to investigate the effect of cabbage cultivar and frequency of insecticide application on cabbage looper, *Trichoplusia ni* (Hübner), damage in both the spring and fall seasons. The mean number of frame and wrapper leaves varied significantly among the cultivars, and the number of days to harvest also differed. Both insecticide frequency and cultivar had significant effects on the amount of cabbage looper damage to the leaves (percent leaf damage and leaf rating) and heads (percent damage) of ten cultivars. In considering both leaf and head damage over both seasons, 'Rio Verde', 'Super Market', 'Superette', and 'Gourmet' sustained the least amount of damage. The interactions between cultivar and insecticide frequency for both seasons were not significant, indicating that the ten cultivars responded to the three levels of insecticide frequency in a similar manner. There was an additive effect of cabbage resistance and insecticide, where the resistant cultivars performed the best regardless of the frequency of pesticide application.

RESUMEN

Se hicieron pruebas de campo para investigar el efecto de la variedad de col y la


1A
1B
1C
1D