# BIOLOGY AND ECOLOGY OF <u>DIAPHANIA</u> unionalis (Hübner), AND COMPARATIVE MORPHOLOGY OF D. unionalis

(Hübner), D. hyalinata (Linnaeus),

AND D. nitidalis (Stoll)

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### INTRODUCTION

Cultivation of the United Arab Republic deserts is one of the main constructive projects recently started in the U.A.R. One of the desert areas chosen for this purpose is the North Western Coast (The Mediterranean coastal area) which extends for about 600 Km from Alexandria to the town of Sallum on the Libian border.

In this region, different types of plants including almonds, figs, apples, pears, peaches, and olives have been introduced. Of these types, the olive trees grow most successfully, as they require the least amount of water and can tolerate salinity. The meteorological conditions and particularly the amount of rainfall of this region seems to be more favorable for olives than for other trees. This fact encouraged planting of olives in this area, where irrigation water is very costly. Because of these advantages the olive crop is considered the number one crop for the newly reclaimed desert land.

Known insect infestations of <u>Oleae</u> sp. in this region included scale insects (order Homoptera) and the olive fly, <u>Dacus oleae</u> (order Diptera). Later it was observed that the yield of olives was decreasing year after year, though the agricultural practices were the same. A detailed study of all parts of the trees in the olive plantations of the region indicated that two lepidopterous larvae were causing defoliation

and fruit damage. All stages of these pests were collected and reared under laboratory conditions on grafted seedlings of <u>Oleae europea</u> var. chemlali. Specimens of the two moths were sent to the British Museum of Natural History and the U.A.R. collection at the Ministry of Agriculture, Entomology Department.

These two insects were identified as <u>Palpita unionalis</u>, Hbn. and Zelleria oleastrella, Mill.

Biological and morphological studies of these pests were initiated in the field and laboratory.

This study will provide new information about the importance of these two pests to olive and jasmin plantings. The morphology and biology of <u>D. unionalis</u> will be compared with those of two important pests in the United States which belong to the same genus; <u>D. nitidalis</u>, (Stoll) and D. hyalinata, (Linnaeus).

### **REVIEW OF LITERATURE**

### Genus Diaphania

The nomenclature of <u>Diaphania</u> has been complex and is still in doubt. In literature, reference is made to at least 12 names for the genus now known as <u>Diaphania</u>. Of these only <u>Diaphania</u>, <u>Palpita</u>, <u>Hapalia</u>, <u>Conchia</u>, <u>Margaronia</u> and <u>Glyphodes</u> have been widely used. Munroe (1950) stated that the fairly well known name, <u>Margaronia</u> must fall to the unfamiliar <u>Palpita</u>. <u>Margaronia</u> was, however, antedated by three names. It had generally been used for a large and composite group of species, of which <u>Palpita</u> as defined there included only a small part. Even in that sense the usage was by no means general. He added also that there was little ground for proposing <u>Margaronia</u> as a nomen conservandum, and Palpita must accordingly stand.

Kuchlein (1958) stated that the well known name <u>Margaronia</u> "a dust bin for hundreds of tropical and subtropical species", must fall for it was preceded by three other names of the same application in Hubner's own works, viz, <u>Palpita</u>, <u>Hapalia</u> and <u>Conchia</u>. <u>Palpita</u> was the oldest name and as defined by Munroe it included only a small part of the group assembled in the genus Margaronia.

### Biology of D. unionalis Hbn.

Martilli (1915) found that in southern Italy there were at least five generations a year, the life cycle varied from 33 to 82 days according to season. He also added that <u>Palpita unionalis</u> was seen flying around the olive branches with new growth, and when disturbed by day it flew away zigzag and settled quickly under another leaf.

Riba Ferré (1920) stated that the caterpillar of <u>Glyphodes</u> (<u>Margarodes</u>) <u>unionalis</u> was difficult to combat because it made a shelter within a case made of leaves. On the other hand Berland and Seguy (1922) found that <u>Glyphodes unionalis</u> Hbn. caused important damage to jasmin cultivation in the Cute Dozie area (South of France) but it also attacked olive and troa. They described the feeding habits of the larvae, gave the length of the full grown larvae as 2 - 2.5 cm and stated that pupal stage occurred in a white cocoon formed in the fold of a leaf or between two leaves.

Malenotti (1926) observed two generations; one occurred in August and the other one in September. The pupal stage lasted 11 days in the former and 16 days in the latter. La Vida Agricola (1931) mentioned that a Pyralid, <u>Diaphania</u> sp. occurred in almost all olive plantations in Peru, and in 1930 caused serious crop losses (up to 60 percent) in the Ilo Valley. Activity began in April or May, reached its maximum in August and September, and ceased during the summer (December - March). The larval and pupal stages lasted about 3 weeks, and 2 weeks respectively. The eggs were laid and the larvae fed on the

shoots, young leaves, peduncles of the flowers, and fruits.

Balachowsky and Mesnil (1936) described the larval habitat on jasmin in Southern France. They added also that the flowers were attacked. Morris (1937) found that <u>Margaronia</u> (<u>Glyphodes</u>) <u>unionalis</u>, Hbn. was a pest on young shoots of olive trees in Cyprus.

Howarth (1950) studied the biology of <u>D</u>. <u>unionalis</u> on <u>Jasminum</u> officinale (white jasmin). He found four instars. The cocoon was made of a tough white silk on the outside with an inner fabric of finer regularly crossed silken strands. The larvae turned to pupae within two or three days after spinning their cocoons. Two generations were found, the duration of the first was 36 days and the second 31 days. Kalshoven (1950) figured an attacked leaf at the tip of jasmin. He remarked that in Java the pest developed especially at the end of the rainy season and might continue for several months. Complete defoliation often occurred.

Fahmy (1953) stated that although <u>Glyphodes unionalis</u> Hbn. had long been collected around lights in Marsa Matrouh, the Mariout, Ramleh (Alexandria), Helwan, Maadi, and Koubbeh. Damage to olives was first noted in 1931 in the Mariout district, but little was known at that time concerning the damage caused to olive trees by this pest of Oleinae, which previously had been recorded as a pest of jasmin and olive in other countries. He also added that its distribution was Mediterranean and that it also occurred in Mauritania, Madeira, Canary Island and in the Indo-Australian region.

Bretherton (1955) mentioned that in the daytime in England the moth had only been found at rest. It appeared to fly at dusk, and fed on ivy, Arbutus, honeysuckle, Buddleya or on sugar.

### Morphology of D. unionalis

Berland and Seguy (1922) provided a brief description of the larval and adult characteristics of <u>D</u>. <u>unionalis</u>. Malenotti (1926) working in Italy stated that the butterfly <u>Diaphania</u> (<u>Margaronia</u>) <u>unionalis</u> measured approximately 24 mm when the wings are open in contrast to the 18-22 mm reported by Berland and Seguy. He also reported a more definite pattern on the wings. Balachowsky and Mesnil (1936) mentioned that the full-grown larva of <u>Diaphania</u> (<u>Glyphodes</u>) <u>unionalis</u> is 20 to 25 mm long, possesses an apparent longitudinal dorsal line and the pseudopodia are well developed.

Howarth (1950) provided brief descriptions of the eggs, larval instars and pupae. Munroe (1950) described the genus <u>Diaphania</u> (<u>Palpita</u>) Hubner; the type used in the study was <u>Palpita unionalis</u> Hbn. He also described male and female adults, the wing venation, and the genitalia.

Nazmi (1963) described the general appearance of the moth, the head, thorax, abdomen, and male and female genitalia.

### Morphology of D. nitidalis and D. hyalinata

Smith (1911) followed the work of Quaintance (1901) in describing the stages of the pickleworm. The adult stage has a general yellowish

brown color for the body and wings with an irridescent purplish reflection. He described both sexes and said that the abdomen in them terminates in a prominent brush, which is small in the female and more curved in the male. In the same paper he also described the different stages of the melonworm Diaphania hyalinata. He described the egg stage, its length, and color. The larval instars were described, and the full-grown larvae varied from 1.9 to 3.2 cm. Smith mentioned that if the melonworm larva is taken just prior to pupation, it can be distinguished from the pickleworm, by having a much lighter brown head, longer and more slender setae, rather more slender body and no sign of coppery color above. The pupal stage has the head rather acutely pointed, somewhat flattened dorsoventrally, caudal end acute and bearing a group of a few stiff spines with strongly curved tips. A comparison of the melonworm pupae with those of the pickleworm indicated that the former have a longer and more flattened head and longer leg shields, they also present a rather more slender appearance, but on the whole the two species are very similar.

Fulton (1947) gave a general description for the pickleworm moths, different larval instars, the prepupal stage, and the pupal stage.

Reid and Cuthbert (1956) stated that the adults of the pickleworm are pretty moths that are easily distinguished from other insects with the possible exception of the melonworm. They gave a full description of all stages. They also mentioned that the posterior end of the pupa

is pointed and bears several spines which have curved tips that hold the pupa in its thin cocoon.

Peterson (1962) clearly described the external morphology of Diaphania nitidalis Stoll, (pickleworm). He stated that the full-grown larvae measured from 25-30 mm, were greenish to yellowish-green, without the pigmented pinacula typical of earlier instars especially the fourth. Head yellowish brown with a distinct pigmented spot on the lower half of its caudal margin. Seta Kappa (4) on eighth abdominal segment, two or more times as far away from the spiracle as the greatest diameter of the spiracle. Mandibles with five dentes extending over the entire distal margin. He also found that the full-grown larvae of Diaphania hyalinata Linn., (melonworm) measured 25 to 30 mm, and was greenish to yellow resembling the full-grown larvae of D. nitidalis. Earlier instars without pigmented pinacula and possessed two slender white stripes on the dorsomeson. Head yellowish-brown and without a pigment spot on the caudal margin. Seta kappa (4) on the eighth abdominal segment approximately the same distance from the spiracle as the greatest diameter of the spiracle. Mandibles with five dentes not covering the entire distal margin, an additional small projection present on the distolateral margin.

### MATERIALS AND METHODS

Collection

### LARVAE

Larvae of D. unionalis and Z. oleastrella were collected from infested olive trees at Burg El-Arab. Collection always started in the morning about 6 AM, while the larvae were feeding on different parts of the plant. The infested part was cut below the place where the larvae (different instars) were feeding by the use of pruning shears. The small branch was sealed immediately by Alexandrian wax and put into a glass jar that contained some newly cut and sealed uninfested terminal buds for feeding. The jar was then covered with muslin. The eggs of D. unionalis that were deposited on the leaves were also collected. Collection was made from different trees and monthly. The larvae of Z. oleastrella were collected in the same manner. Mostly found feeding on the flowers and new buds, larvae of D. unionalis were also collected from the fruits of olive trees of several varieties where the larvae tunnelled extensively. The infestation was readily visible especially where the larvae tunnelled from one fruit to the next. The infested branch in this case was also cut below the infested part, sealed with wax, and put in the jar.

### PUPAE

The pupae of <u>D</u>. <u>unionalis</u> and <u>Z</u>. <u>oleastrella</u> always were found in defoliated leaves beneath the tree or in close association with the trunk. The pupae of <u>D</u>. <u>unionalis</u> were found within clusters of defoliated leaves, sometimes inside one rolled leaf, and occasionally between two attached leaves which were secured by silken threads and surrounded the pupa completely. The defoliated leaves containing pupae were collected separately in different jars and covered with muslin secured with a rubber band. Some of the pupae were found in the moist sand surrounding the trunk. Most of the pupation of <u>Diaphania</u> on olive trees occurred on the lower part of the tree. A few were found on the large branches. Pupae of <u>Zelleria</u> were also found within the folds of the leaves. The pupae of both insects were collected separately by cutting the infested part, sealing the branch, and placing it into a glass jar covered with muslin.

### Sampling

During November 1962 experimental plots were selected at Burg El-Arab (50 Kilometers from Alexandria along the Mediterranean coast), to study the infestation rate in buds of <u>Oleae europea</u> var. Chemlali, the most dominant variety cultivated in the area. This area involved approximately 54,600 m<sup>2</sup> and was not irrigated or fertilized except in 1962 decorticated cotton seed meal (Two Kgs per tree) was added. The orchards were established in 1927. Five grafted trees

similar in age and height and of the variety Chemlali were selected at random. A buffer zone of at least four rows of trees surrounded each plot. Each tree was divided into three levels: low, middle, and high and North, South, East and West quadrants. Ten new uninfested terminal buds were chosen from each quadrant on different branches and were distributed from the inside to the outside of the tree, and were colored with different colors in each side and were similar in every level in each tree. These trees were numbered from 1 to 5 and the trunk of each was colored with white paint to approximately 50 centimeters above ground level. At the beginning of the third week of each month during the period starting January 1963 until December 1963, from January 1964 until December 1964, and from January 1965 until May 1965, these marked terminal buds were examined thoroughly and the numbers of infested and uninfested buds were recorded. The type of infestation was taken into consideration. This means that the flower, fruit infestation, or leaf infestations were observed and recorded. Maximum, minimum and average daily temperature, relative humidity, rainfall, and wind velocity were recorded daily.

### Rearing

All insect rearing was done in the laboratory under known conditions. Temperature and humidity conditions were recorded at all times. During the daylight hours illumination was provided by windows covering half of the wall space on the north and west sides supplemented by fluorescent lights. During the night low intensity lights were used.

After collecting the specimens from the field, they were taken to the laboratory and placed in a large cage 181 x 132 x 51 cm covered with screen wire. The cage rested on a bench and its legs, while were about 12 cm in length, stood in zinc containers filled with water. The stock culture was supplied by 10-18 small pots which contained grafted one-year old seedlings of <u>Oleae europea</u> var Chemlali. A piece of cotton, wetted by 8% sugar solution, was placed in a small Petri dish covered by filter paper. Water was provided in the same manner. A small amount of dry sugar was put in a third small container. These containers were changed daily. The small pots inside the cages were irrigated every other day with 50 ml distilled water for each pot.

Leaves bearing eggs less than 24 hours of age were secured to a small seedling to initiate an infestation. Twenty eggs were used for each seedling. The seedlings were kept in cages 133 x 40 x 50 cm covered with metal screen wire and rested on benches whose legs stood in zinc containers filled with water to avoid predation. Distilled water was used for irrigation.

Newly hatched larvae were reared in small glass tubes (76 x 32 mm) with the ends covered with muslin. Each day larvae were fed new growth olive twigs with the cut ends dipped in wax. The larvae were examined daily and the head capsule width and length of each larva were measured after each molt.

The pupae were collected from the stock-culture and each was put in a small tube provided with new growth of olives. These tubes

were examined each day to obtain males and females of the same age.

Males and females of the same age were maintained in cages similar to those used in the larval studies to study the number of eggs laid by each female, duration of the egg stage, and hatching. A male and a female were put in a glass jar, 32 x 18.3 cm, provided with an olive branch, sugar solution, and water to determine the periods between emergence and mating, and between mating and oviposition. This jar was observed continuously.

To determine survival time of newly emerged males and females, individuals were placed alone in small jars without food or water. Each day survival was recorded.

Grafted olive seedlings one year old were used as host plants. Also Jasminum sp. was used.

#### Preparation For Examination

Eggs were prepared for examination by mounting directly in Down's medium which consisted of:

phenol 22 g, lactic acid 22 g, and

polyvinyl alcohol 56 g.

Larvae from different instars of <u>D</u>. <u>unionalis</u> reared in the laboratory were placed in boiling water for 10 seconds, removed, and injected in the ventro-lateral aspect of the abdomen above the third abdominal leg with a fixing solution. It consisted of:

5 parts formaldehyde, 5 parts glacial acetic acid, and 90 parts 70% ethyl alcohol.

The larvae were put in small tubes containing the same solution for a day and then transferred to fresh solution for permanent preservation.

Larvae and pupae of <u>D. hyalinata</u> and <u>D. nitidalis</u> were obtained from Clemson, South Carolina. Also larval and pupal samples of <u>D.</u> <u>nitidalis</u> were obtained from the Department of Entomology, University of Georgia. All of these samples were preserved in 80% ethyl alcohol.

Pupae of <u>D</u>. <u>unionalis</u> were obtained from laboratory rearing and were preserved in the same liquid used for preservation of laboratory reared larvae.

Pupae of <u>D</u>. <u>nitidalis</u> and <u>D</u>. <u>hyalinata</u> obtained from Clemson University and the University of Georgia were preserved in 80% ethyl alcohol. Adults reared in the laboratory were pinned, spread, and stored in wooden boxes. No adult moths of <u>D</u>. <u>nitidalis</u> or <u>D</u>. <u>hyalinata</u> were available.

Permount medium was used in preparing permanent slides for the study of morphological differences in the mouth parts. After separating the mandibles from the head they were transferred to 95% ethyl alcohol for a few minutes, carbol-xylol for 1/2 hour, washed briefly with toluene, and mounted in Permount.

### **RESULTS AND DISCUSSION**

### Distribution

The distribution of the important economic species of <u>Diaphania</u> has been clearly delineated. <u>D. unionalis</u> is essentially world wide, wherever the host plants are found. In the United Arab Republic in 1962 the author found <u>D. unionalis</u> causing economic damage to olives (Fig. 1) in the following areas: In Wadi El-Natron, the survey showed the presence of <u>D. unionalis</u> and <u>Z. oleastrella</u> on olive trees. In El-Arish region (N.E. U.A.R.) <u>D. unionalis</u> caused severe damage to olive plantations. Infestation of <u>Zelleria</u> was light. In Gaza region the infestation of olives was mainly by <u>Zelleria</u> and damage by <u>Diaphania</u> was not as severe. In the North Western Coastal region starting 20 Km west of Alexandria and extending for 40 Km along the coast, the infestation of <u>Diaphania</u> was observed at the Arab farms scattered in that area.

In Mariout region infestation was by <u>Diaphania</u>. In Burg El-Arab region infestations of both <u>Diaphania</u> and <u>Zelleria</u> were observed. In Foka region the infestation of olives by <u>Diaphania</u> and <u>Zelleria</u> was severe particularly in irrigated areas. In Ras El-Hekma region the olive trees in the different farms were severely infested by <u>Diaphania</u> and Zelleria. In Marsa Matrouh region 280 Km along the Mediterranean

Coast, the infestations by the two insects were very apparent on the different varieties of olive.

In Sewa oasis olive trees were also infested by both insects. In El Baharya region infestation of olives was mainly by Diaphania. The percent of terminal buds infested by D. unionalis and Z. oleastrella for 1963 is shown in Table II. The rate increased as the temperature and humidity increased (Fig. 2). The infestations by both insects started during the third week of May. No infestations occurred after October when the temperature and humidity started to decrease. It should also be noted that the range of relative humidity was very small, 61 - 73% R.H. When the temperatures began to decrease the infestation stopped and the insect hibernated until the next season. During 1964 the infestation of the terminal buds by D. unionalis started in the third week of May (Table II), while the infestation by Z. oleastrella started during the third week of April. This shows that activity of Z. oleastrella begins earlier than that of D. unionalis. Fig. 3 shows that in D. unionalis the infestation rate increased as the temperature increased, but it also reached its maximum during October though the maximum temperature occurred in August. The increase was due to the activity of the last generation of the season after which the insect hibernated. The rate of infestation also increased within the range of relative humidity observed (63 - 70% R.H.). The infestation rate of Z. oleastrella started during April, increased until it reached its maximum in September and ceased while the temperatures were

### TABLE I

### Average monthly climatic factors of Burg El-Arab region during 1962, 1963, and 1964.

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
		,		Max	imum a	ir tempe	erature	C				
1962	19.0	17.7	22.9	23.0	24.0	28.5	29.1	30.8	29.4	27.6	28.1	19.5
1963	19.5	20.2	20.5	22.4	25.9	27.1	29.3	30.1	29.3	27.7	23.7	21.1
1964	17.3	18.3	21.3	22.2	24.9	27.9	28.9	29.8	27.8	27.0	21.6	19.4
				Mini	.mum ai	r tempe	rature	C				
1962	10.7	9.2	13.5	14.5	16.9	21.1	22.9	23.6	22.7	19.2	17.3	10.8
1963	9.6	10.3	11.3	14.5	16.9	19.8	23.5	23.5	22.6	19.3	15.8	11.0
1964	8.9	9.5	12.6	13.9	15.4	20.3	22.8	23.5	21.7	18.0	14.7	11.4
				D	aily air	temper	ature C	<u>,</u>				
1962	14.6	13.7	17.5	18.4	20.9	24.6	26.0	27.2	26.0	23.4	21.7	15.4
1963	14.4	15.1	15.3	18.2	20.8	23.3	26.1	26.6	25.8	23.5	19.9	15.8
1964	13.0	13.9	16.5	17.8	20.3	24.0	25.7	26.4	24.6	22.6	18.9	15.5
					Relativ	e humid	lity %					
1962	65.0	60.0	59	60	66	67	70	68	66	68	74	: 60
1963	64	61	62	69	62	72	73	70	66	69	65	61
1964	66	71	67	63	63	70	70	69	63	69	68	70
				·				·				

### TABLE II

Month	Percent of terminal buds infested by Z. oleastrella	Percent of terminal buds infested by D. unionalis	Percent of terminal but infested by Z.oleastrella	
· · · · · · · · · · · · · · · · · · ·	and <u>D.</u> <u>unionalis</u> in 1963	in 1964	in 1964	
January	0	0	0	
February	0	0	0	
March	0	0	0	
April	0	0	4.2	
May	6.8	8.8	7.5	
June	26.7	21.2	12.8	
July	37.6	32.1	18.6	
August	49.7	41.5	26.5	
Septembe	r 59.8	52.8	34.0	
October	63.9	61.9	0	
November	0	0	0	
December	0	0	0	

Percent of terminal buds infested by  $\underline{D}$ . unionalis and Z. oleastrella in 1963 and 1964

#### decreasing.

The increase of the infestation after the maximum temperature in August explained the activity of the last generation of this insect in the season as it changed oviposition and feeding activity.

It was observed that Z. oleastrella infestations of the olive were in most cases flower infestations. The larvae fed on the flowers first and fed on the buds after destroying the flowers. D. unionalis preferred to feed on terminal buds, fruits, and other parts of the shoots, often causing complete destruction to the new shoots. In a very few cases larvae were seen feeding on flowers. While Z. oleastrella preferred flowers, it also fed on the upper surface of the first two small leaves surrounding the bud. D. unionalis in some cases after feeding on the new buds was seen feeding on the succulent stems. This was not observed in Zelleria infestation. In the trees examined it was observed that the larvae of **D**. unionalis infested the olive fruits during any stage of growth. The larvae tunneled in the fruit on the endocarp and in most cases there were in excess of three larvae inside a fruit. The larvae made many holes in the fruit which encouraged secondary invasion by the black fungi.

Infestation of <u>Zelleria</u> of the fruits was never observed. The larvae of <u>D. unionalis</u> infested fruits of all kinds of olive trees, with no evidence of preference.

### Biology of D. unionalis

#### EGG STAGE

Females of <u>D</u>. <u>unionalis</u> laid their eggs on the lower and upper surfaces of olive leaves, in most cases beside the mid-rib. One to three eggs were usually laid in batches on the left and right of the dorsal vein or were laid in one mass. Eggs were laid also on the succulent petioles.

Howarth (1950) mentioned that the color of the eggs was a creamy white later changing to a pale yellow, but in all cases we noted the color was greenish yellow during the first two days and then changed to orange color with small red points appearing just before hatching. After hatching the color changed to whitish grey. The fertilized eggs of <u>D. unionalis</u> (Fig. 4) were elongate oval, the average length of the eggs measured 0.66 mm, and the average width 0.46 mm. Unfertilized eggs (Fig. 5) were 0.44 x 0.4 mm based on measurements of 50 eggs.

From Table III it is evident that the average preoviposition period was 3.2 days. The average number of eggs laid by a female was 282.2. They were laid at irregular intervals during a 5 day period. In cage 7 it can be observed that a minimum number of eggs were laid apparently due to the inactivity of the male. The average incubation period of the eggs was 3.1 days.

Males lived approximately 19.4 days, while females lived about 13.8 days.

	Pre-oviposition	Total no.	Average		· · · · ·
Cage no.	period in day <b>s</b>	of egg <b>s</b> laid	incubation period in day <b>s</b>	Ag Male	e (days) Female
110.	uays		period in days		r ettiare
1 1	<b>3.0</b>	351	3.0	17	11
2	3.0	382	3.25	26	16
3	3.25	378	3.00	20	17
4	3.0	286	2.75	16	18
5	3.0	271	3.50	22	11
6	3.75	268	3.0	18	17
7	3.50	129	3.0	17	12
8	3.0	253	3.25	19	12
9	3.50	246	3.25	18	13
10	3.0	260	3.0	21	11
Average	3.2	282.2	3.1	19.4	13.

### TABLE III

Number of eggs laid by <u>D</u>. <u>unionalis</u> during July and August 1963 when the temperatures average 24,6 C and 60.7% R.H. The three newly hatched females of <u>D</u>. <u>unionalis</u> which were put in separate cages and supplied with sugar solution and olive seedlings laid from 17 - 40 unfertilized eggs at irregular intervals over a 10 -14 day period.

### LARVAL STAGE

#### First Larval Instar

The first instar larvae emerged from the operculate eggs when they were approximately 1 mm in length (Fig. 6), had a wide bright yellow head with darkly pigmented eyes and apparent setae. The head was relatively much larger than the body. Four to ten hours later the larvae reached the terminal buds and began feeding. They were very light and any current of air made them fall from their place suspended by a very thin thread.

In other cases they were found on the under surfaces of the leaves where they tied the margins together to provide a shelter feeding area or they fed on the upper surface of the newly formed leaves surrounding the bud. While the area of feeding is readily seen, it is covered by silken threads and debris. This feeding provided an excellent means of recognizing infestations.

The first instar larvae are golden yellow and the head is very big in relation to the body.

#### Second Larval Instar

The length of second instar larvae is about 3.00 mm and the width

### TABLE IV

# Measurements of the larval instars of <u>D.</u> unionalis Hbn.

Instar	Length of larvae in mm <sup>*</sup>	Width of head capsule in mm <sup>*</sup>
1	1.00	0.4
2	3.00	0.6
3	6.00	0.8
4	9.6	1.2
5	13.5	1.7
6	16.4	2.4
7	22.0	3.5

Measurements indicate the average of 100 individuals.

>¦<

of the head capsule approximately 0.6 mm. The setae of the head and body are easily seen. The feeding areas on the leaves are larger but similar in pattern to that of the first instar (Fig. 7). At times the larvae attach the lower surface of one leaf to the upper surface of another by threads and debris.

### Third Larval Instar

The average length of the larvae is 6.00 mm and the color of the head somewhat darker than in the earlier instars. The body has become a yellow green. This instar is very active and feeding extends to older leaves causing feeding areas to anastomose.

### Fourth Larval Instar

These very active larvae average about 0.96 cm, the head is dark brown and the darker brown ocelli and mandibles are distinct while the body is green. In contrast to earlier instars the fourth instar larvae consume all of the leaf excepting the central vein. The larva may attack the fruits and terminal buds.

#### Fifth Larval Instar

This instar is green, about 1.35 cm and tunneling of the fruit is characteristic. Defoliation may be extensive.

#### Sixth Larval Instar

This instar averaged 1.64 cm in length and feeds on the terminal buds, fruits, and other surrounding leaves.

### Seventh Larval Instar

The average length of this instar is 2.2 cm. This is the last instar and the most destructive one. It feeds on leaves of any size, the terminal shoots, and on fruits, especially those growing in groups. It eats the green buds and the thin branches of the developing shoots and skeletonizes the leaves, leaving only the mid-rib (Fig. 8). The entire fruit may be consumed leaving only the seed and extensive debris.

The average length of the full-grown larva is 2.4 - 2.5 cm. The head is dark, with dark brown mandibles and ocelli. The dorsal heart may be easily seen in the greenish body.

### PREPUPAL STAGE

Before pupation, the full-grown larva usually enters a prepupal stage (Fig. 9) and remains quiescent. It does not feed while spinning a white silken cover in the fold of an olive leaf or by pulling two leaves around itself and securing them by silken threads. The larva shortens in length, becomes coppery in color, surrounds itself completely by a cocoon and becomes a pupa. The length of the prepupal stage varies in different generations. Its duration depends on the temperature and relative humidity as shown in Table VI. The duration of the prepupal stage in 1963 in August and September averaged 2 days at 26.6 C and 60.0% R.H., while during October, November and December it lasted 7 days at 15.37 C and 65.5% R.H. In 1964 (Table VII) the average duration of the prepupal stage was found to be 2.25 days at 25.8 C and

### TABLE V

### Average daily temperature and relative humidity by months during 1963 and 1964 in the laboratory, Mataria, Cairo

Months	19	963	1964		
	Temp. C	R.H.%	 Temp. C	R.H.%	
January	11.24	66	 10.88	66	
February	14.06	58	13.77	55	
March	16.43	56	17.20	54	
April	20,78	51	20.48	49	
May	23.00	47	24.61	43	
June	26.80	.53	27.48	49	
July	24.96	60	26.69	61	
August	28.00	61	27.25	62	
September	25.20	58	24.40	60	
October	20.86	63	21.40	62	
November	17.00	68	16.38	66	
December	13, 75	73	12.00	70	
		·			

### TABLE VI

#### Gener-% Average duration of stages in days Pre-Hatching Incubation Larval Total Pre-Sex ratio Time ation Pupal period oviposition Male Female of year pupal 80 3.00 20.0 3.00 7.50 3.00 36.5 10 6/8 - 7/15 lst 6 2nd 70 3.00 18.0 2.75 6.25 3.00 33.0 8 6 7/16 - 8/17 75 3.00 17.0 2.00 7.00 3.25 32.0 10 8/18 - 9/18 3rd 5 60 9.10 3.50 39.6 9/19 - 10/28 4th 3.50 21.3 2.25 8 4 5th 55 4.00 30.5 7.00 33.6 7 4 10/29 and continued during Nov., Dec., and January.

### Mataria, Cairo 1963.

Biology of D. unionalis under laboratory conditions

## TABLE VII

# Biology of <u>D.</u> <u>unionalis</u> under laboratory conditions Mataria, Cairo 1964.

Gener-	% Hatching	Average duration of stages in days								
ation		Incubation period	Larval	Pre- pupal	Pupal	Pre- oviposition	Total		ratio Female	Time e of year
	<u> </u>	portou	· · ·	<u></u>	· · ·					o or year
lst	75	3,00	16.0	2.50	7.00	3.00	31.5	9	6	6/15 - 7/17
2nd	90	3.25	17.0	2.00	7.25	3.00	32.5	11	7	7/18 - 8/19
3rd	60	3.00	18.2	2.25	8.00	3.25	34.7	7	5	8/20 - 9/23
4th	55	3.75	22.5	3.00	10.25	3.50	43.0	7	4	9/24 - 11/5
5th	60	3. 75	32.0	9.00	36.0			7		11/6 and continued dur- ing Dec. and
	· · · ·	·	••• •••	 <u></u>	·	·				Jan.

60.9% R.H. in the third generation that started on the 20th of August and ended the 23rd of September, while it reached 9 days at 14.3C and 68.19% R.H.

#### PUPAL STAGE

The pupa in its very early stage is green, similar in color to that of the prepupal stage. After one day it turns to light brownish-yellow, and becomes progressively darker, beginning with the caudal end. Within four days the pupa has become a uniform dark brown (Fig. 10).

The duration of the pupal stage varied in each generation depending on temperature and humidity. The average duration of the third generation pupal stage, which began August 18 and ended September 18, was 7 days (Table VI). Fifth generation pupae appeared first on October 29 and extended through the winter months. The average duration of the pupal stage of the fifth generation was 33.6 days during 1963. In 1964 the average duration of the pupal stage was 8.00 days at 25.8 C and 61% R.H. in the third generation and 36 days at 14.25 C and 68% R.H. in the fifth generation.

#### ADULT STAGE

When hatching from the pupa, the adult emerged from a T-shaped opening over the dorsum of the head and thorax (Fig. 11).

The adult moths are greyish white dorsally, white ventrally, and with an alar expanse of about 11 mm from the middle of thorax to the apex of the forewing. Males with posterior tuft of long white scales overlaid with black scales.

Emerged males always outnumbered females; regardless of the time of year by 25 to 50%. Adults were relatively inactive from January to May.

#### Longevity of the Adults

The average number of days both males and females (average taken from five individuals of each sex) survived without food under laboratory conditions was 5 days for the males and 4 days for the females. During the experiment it was noticed that the moths became progressively less active and failed to fly or crawl to the top of the jar.

In the rearing cages longevity was greatly increased. Males lived about 19 days and the females about 14 days.

#### Mating and Oviposition

The experiment was begun at 8 AM with moths which had emerged between midnight and 8 AM. During the first day both sexes began to move their wings up and down rapidly, flying in different directions in the jar for a few minutes, standing far from each other and no mating was observed but feeding on sugar solution was common.

On the second day feeding continued and the first mating dance occurred 43.5 hours after the experiment started. Both male and female were standing in opposite direction for half an hour, wings were extended, and the antennae were extended anteriorly. On the third day, 23 hours after mating, the female was observed laying eggs in small batches on the midrib and a few on the upper surface. After an hour no more eggs were laid, no additional matings were observed and the male was standing far from the female. On the fourth day no mating was observed and the male and female were standing far from each other. The female began again to lay eggs on the leaves. After another day more eggs were laid. The female died after 10 days, and the male died after 16 days.

This experiment was done in August 1964 at 27.3 C and .62% R.H.

#### NUMBER OF GENERATIONS

Five generations were completed each year under laboratory conditions. Temperature and humidity were the major determining factors governing the duration of each generation as indicated in Table VI. The third generation, the shortest, was completed in 32 days while the fifth required over two months. In 1964 the period was 34.70 days in the third generation at 25.8 C and 61.0% R.H., while it lasted more than 2.5 months in the last generation at 14.25 C and 68.2% R.H.

#### Effect On The Host

From the studies made in the field at Burg El-Arab, it was found that <u>Z</u>. <u>oleastrella</u> infested the plant early in April in the flowering stage, fed on the flowers mainly and on the small leaves that surrounded the new buds (Fig. 12). This infestation destroyed many flowers and resulted in marked reduction of the olive crop. <u>D. unionalis</u> infestation of the olive included the invasion of terminal shoots by younger instars which resulted in severe damage to the fruiting wood and the marked reduction of fruit yield (Fig. 13). Later instars fed on the leaves and adventitious buds and reduced the amount of foliage. In addition, fifth and later instar larvae moved to developing fruit and usually made it unmarketable (Fig. 14 and 15). Due to the infestation by these two pests the olive crop was reduced 70 - 80% in 1964.

#### Host Plants

Olive plants were the preferred hosts for <u>D</u>. <u>unionalis</u>. Jasmin, which may be heavily attacked in France (Berland and Seguy 1922), was much less attractive than olives under laboratory conditions. Moths preferred olive plants for egg oviposition and larvae failed to complete growth on jasmin. Larvae given equal access to olive and jasmin leaves preferred olive leaves. The insects preferred grafted plants to seedling plants for the former have soft leaves which are large and have a great number of soft shoots and bear more fruits while the latter have short leaves which are very hard and also the shoots are very hard and spiny and carry less fruit.

During the survey made in the different regions, it was observed that the irrigated trees are severely infested by the two insects, particularly <u>Diaphania</u>, this was very clear at Foka region. It was observed at Marsa-Matrouh region that the grafted seedlings were infested from the mother tree during the grafting period and these seedlings were completely destroyed when the larvae of <u>Diaphania</u> ate the terminal shoots.

#### Morphology of D. unionalis, Hbn.

#### ADULTS

Adults of <u>D</u>. <u>unionalis</u>: wing spread about 22 mm, wings white with a greenish tinge when first emerged, within 24 - 48 hours become grayish-white, general color of the body, white (Fig. 16). <u>Head</u>: Frons rounded; maxillary palpi long, ending in a flat tuft of scales. Behind the eyes a row of very light yellow scales; labial palpus porrect with fine brown scales dorsally, white scales ventrally. Antennae filiform, grayish-white pitose.

<u>Thorax</u>: Thorax white dorsally and ventrally. Wings are more or less triangular; apices rounded; frenulum absent; costal margin brownish dorsally, light yellow ventrally. Wing venation (Fig. 17) normal for Pyralidae; Sc nearly two-thirds length of wing;  $R_1$  arising at or before middle of the cell; stalk of  $R_3 + 4$  longer than free portions;  $R_5$  bent and approximated at the base to  $R_3 + 4$ ;  $M_1$  close to  $R_5$  at the base; parallel distally;  $M_2$ ,  $M_3$  and  $Cu_1$  close approximate at base, divergent distally;  $Cu_2$  arising from distal fourth of cell, 2nd A reaches margin, 3rd A less than one-third length of wing. Anal loop large discocellular moderately curved in both fore and hind wing. The hind wing same color as fore wing, Sc little more than one-half length of wing;  $M_1$  from cell more than one-half length of wing; lst, 2nd, and

#### 3rd A well developed.

<u>Abdomen</u>: Males easily distinguished from females by a posterior tuft of long white scales overlaid with black scales (Fig. 18). Abdomen white dorsally with darker scales at the posterior margin of segment, white ventrally. Legs with all coxae white, tibia and spines of tarsal segments brown.

#### LARVAE

There are 7 larval instars in <u>D</u>. <u>unionalis</u> which change appreciably in appearance. Major changes are in maculations and relative proportion of the body length (Fig. 19).

#### First Instar

Length about 1.0 mm, head relatively large in relation to the body. Color of body golden yellow, ocelli and mandibles brownish, head subcordate, about 0.4 mm wide; prothoracic shield moderately distinct; thoracic legs well developed. Prolegs normal, with triordinal crochets. Anal prolegs present, normal. Setae approximate arising from moderate tubercles, apparent.

#### Second Instar

Length about 3.0 mm. Head light brown, ocelli and mandibles dark brown. Color of body as in first instar. Head, about 0.6 mm wide. Tubercles as in first instar, setae not close together as in first instar.

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#### Third Instar

Length about 6.0 mm. Head about 0.8 mm. Ocelli, mandibles dark brown. Large brown maculations surrounding the tubercles on the three thoracic segments and the eighth abdominal segment well developed. Color of body greenish yellow. Setae more apparent.

#### Fourth Instar

Length about 0.96 cm. Head about 1.2 mm wide. Color of body green. Maculations surrounding the tubercles more distinct and occurring in more or less transverse rows across the body; found on all thoracic and abdominal segments except ninth and tenth; more distinct on eighth abdominal segment. Mandibles and ocelli much darker than in previous instars.

#### Fifth Instar

Length about 1.35 cm. Head about 1.7 mm wide. Color of body greenish; the distinct black transverse spots of earlier stages are lost in the molt of the previous instar except in those of the thoracic segments and the eighth abdominal segment. Ocelli and mandibles very dark and noticeable. Setae and tubercles as in earlier instars.

#### Sixth Instar

Length about 1.64 cm. Head 2.4 mm; color of the body greenish. The black transverse spots are found only on the meso and metathorax, and eighth abdominal segment. Ocelli and mandibles dark brown. Dorsal heart visible.

#### Seventh Instar

Length about 2.2 cm. Head about 3.5 mm wide. Color of the body dark green with dark brown mandibles and ocelli. The black transverse spots are found on the thoracic segments and only on the eighth abdominal segment.

Comparative Morphology of the Three Species of <u>Diaphania</u> LARVAE

In <u>D</u>. <u>unionalis</u> the mandible is compact, only four short teeth present. No processes were observed (Fig. 20 A and D).

Mandibles of <u>D. hyalinata</u> with five rounded dentes not encompassing the entire distal margin. An additional small den just above the mandibular setae is characteristic of this species. Mesodistad of the mandibular setae are two protruberences, the most distal is the larger. Mesad of the setae 10 - 20 irregular depressions may be seen (Fig. 20 B and E).

The mandibles of <u>D</u>. <u>nitidalis</u> with five sharp dentes extending over the entire distal margin (Fig. 20 C and F). Distad of the condyle on lateral and mesal aspects are four elongate bosses, decreasing in size distally to proximally. Basal half of lateral and mesal surfaces with small rugae.

In <u>Diaphania</u> spp., on the eighth abdominal segment, the distance between seta kappa (4) and the spiracle constitutes a very important character in separating the three species. In <u>D. unionalis</u>, this distance is about one and a half times the largest diameter of the spiracle (Fig. 21 A). In <u>D. hyalinata</u>, this distance is about the same as the largest diameter of the spiracle (Fig. 21 B). In <u>D. nitidalis</u>, the distance is about two and one half times the largest diameter of the spiracle (Fig. 21 C).

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In <u>D. unionalis</u>, the arrangement of the crochets differs completely from the other two species. Crochets on prolegs are uniserial and triordinal but arranged in complete circle (Fig. 22 A).

In <u>D. hyalinata</u>, crochets on prolegs are uniserial and triordinal, margin sinuate. The arrangement of the crochets and the gap are the same as in D. nitidalis (Fig. 22 B).

In <u>D. nitidalis</u> (Fig. 22 C), crochets on prolegs are uniserial and triordinal, arranged in an incomplete circle, margin regular. The gap in the circle on the lateral aspect is usually less than one-third the projected circumference of the circle.

#### **D.** unionalis

The full grown larvae of <u>D</u>. <u>unionalis</u> measured about 2.5 cm; color of the body greenish with dorsal heart visible (Fig. 23). The head is yellowish brown with a distinct very dark brown to black spot on the posteroventral surface of the gena (Fig. 24 A). Thorax with a yellowish light brown cervical shield; abdomen with prolegs on the third, fourth, fifth, sixth, and the tenth segments. Distinct dark brown to black maculations surrounding the tubercles on the thoracic and eighth abdominal segments.

#### D. hyalinata

Head is light yellow brown without any colored spot on gena (Fig. 24 B). Color of body greenish to yellowish green. Thorax with a brown cervical shield. Abdomen with prolegs as in <u>D. unionalis</u>. No distinct maculations surrounding the tubercles either on thorax or abdomen.

#### D. nitidalis

Head light yellowish brown with a distinct very dark brown to black spot on the posteroventral surface of the gena (Fig. 24 C). Color of body the same as in <u>D. hyalinata</u>. Thorax with a light yellowish brown cervical shield, abdomen with prolegs as in other species of <u>Diaphania</u>. No large maculae surrounding the tubercles either on the thorax or the abdomen.

#### PUPAE

#### D. unionalis

Pupa average 13 - 14 mm in length. The general color is light brown. Leg sheaths reach the distal margin of the fifth abdominal segment. Cephalic end blunt, caudal end acute and bears a group of apparent short hooks. Pupa much shorter in length than the other two species (Fig. 25 A). Spiracles are apparent, setae sparse, arrangement uniform on all abdominal segments. Male pupae smaller than female.

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#### D. hyalinata

Average length 16 to 19 mm, head acutely pointed, somewhat flattened, dorsoventrally. The tip of the abdomen acute, bearing a group of apparent hooks which hold the pupa in its cocoon. Leg sheaths and antennae are much lighter brown than the dark brown head. Spiracles and setae as in <u>D. unionalis</u>. Leg sheaths extending beyond the middle of the 7th abdominal segment (Fig. 25 B). Pupa slender in appearance

#### D. nitidalis

The general color of the pupa is light to reddish brown. The cephalic portion of pupa blunt, tapering to a point caudally, with 5 - 6 hooks at the tip which hold the pupa in its cocoon (Fig. 25 C). The length of the pupa varies from 13 to 19 mm. Spiracles and setae as those in the other two species of <u>Diaphania</u>. Leg sheaths extend to the middle of the 6th abdominal segment.

#### SUMMARY AND CONCLUSIONS

Olive plantations of the north western coastal area and related desert areas of the United Arab Republic were severely infested by <u>Diaphania unionalis</u>, Hbn. Great reduction in the olive crop (more than 60%) was recorded as a result of the infestation by this insect.

<u>Diaphania</u> infested the terminal buds, flowers, leaves, and fruits. Seasonal distribution in relation to climatic factors was studied in the field at Burg El-Arab. Infestation of <u>Diaphania</u> began in mid May and extended through October. The biology of <u>Diaphania</u> was studied in the laboratory under ambient conditions. Five generations were noted. The preoviposition period averaged 3.2 days, and an average of 282 eggs was laid by females at irregular intervals during a 5-day oviposition period. Females kept alone laid 17 - 40 unfertilized eggs at irregular intervals over periods of 10 - 14 days.

The average incubation period was 3.1 days, and the length of life of fed males and females was 19.4 and 13.8 days, respectively.

Seven larval instars were found. Variations in temperature and humidity greatly altered the rate of development of all stages. Measurements of the body length of the larvae and the width of head capsule were recorded. The feeding habits of each instar were studied.

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The longevity of the adults kept under laboratory conditions without food was 5 days in the males and 4 days in the females. The mean period between emergence and mating was 43.5 hours, and the period between mating and oviposition was 23 hours.

The detailed morphology of the different stages of <u>D</u>. <u>unionalis</u> and the comparative morphology of <u>D</u>. <u>unionalis</u>, <u>D</u>. <u>hyalinata</u>, and <u>D</u>. <u>nitidalis</u> were studied. Distinguishing characters of the larvae were found on the head, mandibles, eighth abdominal segment, and crochets. Also the general shape, color, and the length of the leg sheaths of the pupae constituted other characters for separating the three species.

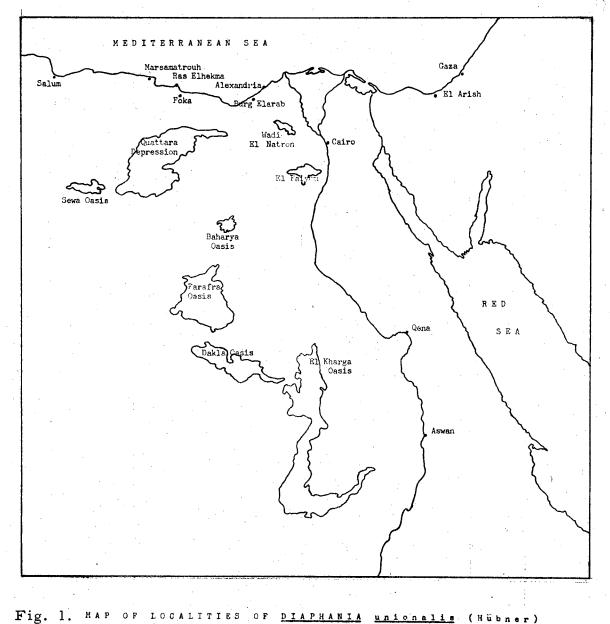
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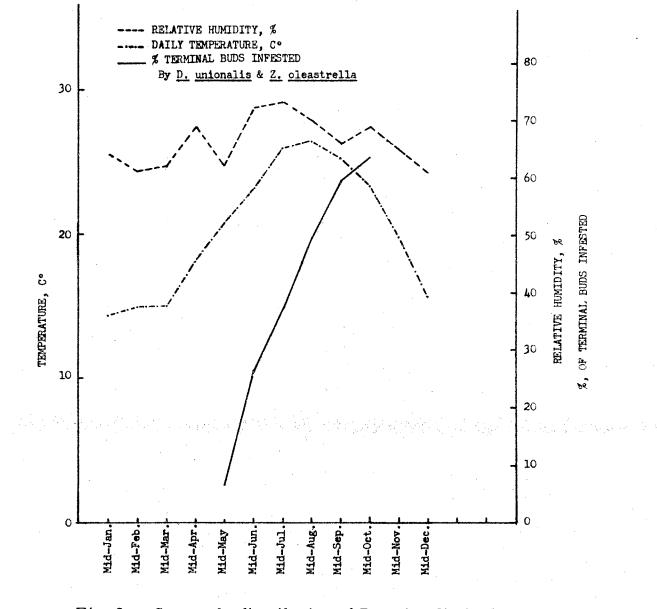
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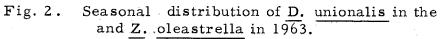
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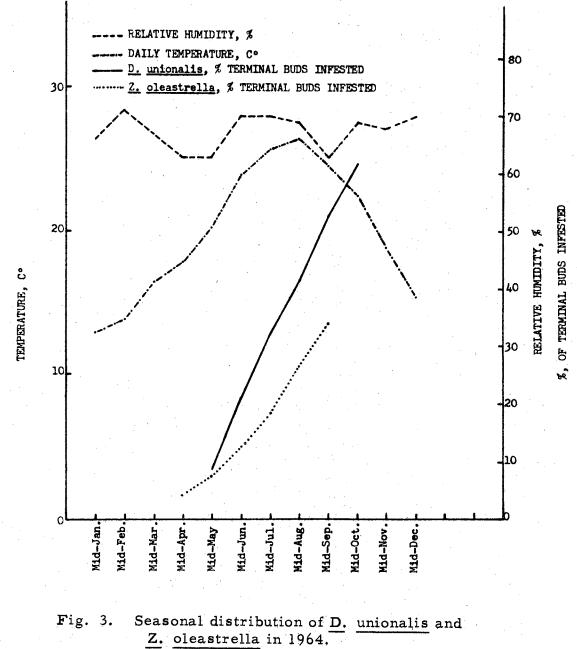
### APPENDIX

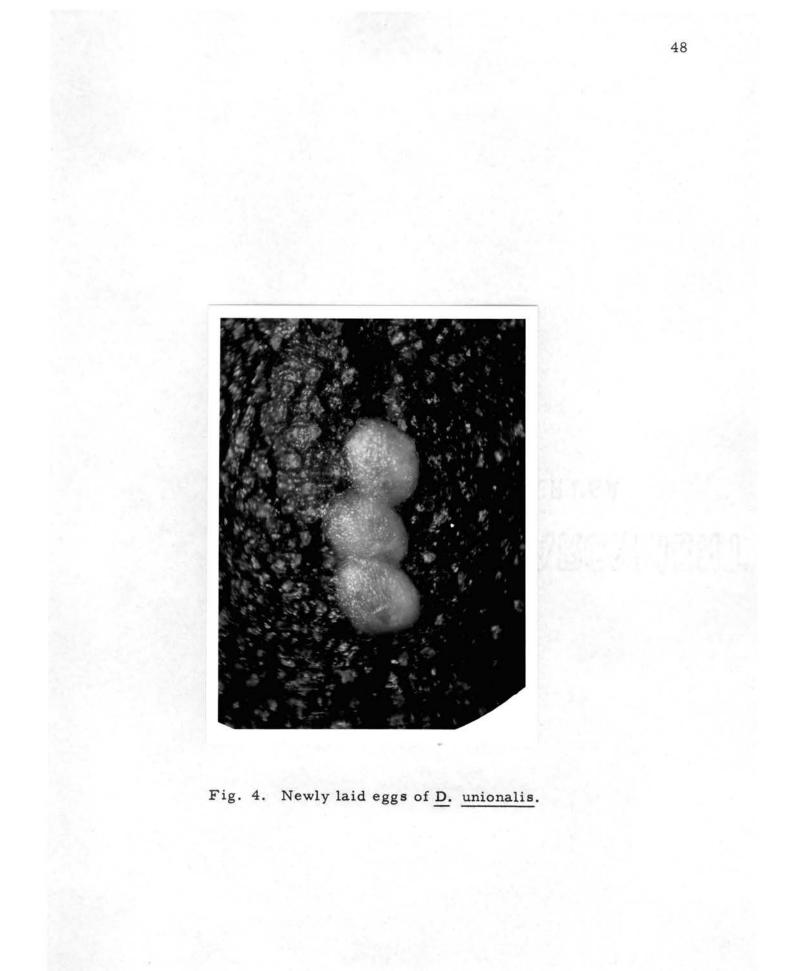


IES OF <u>DIAPHANIA</u> <u>unionalis</u> () IN U.A.R.









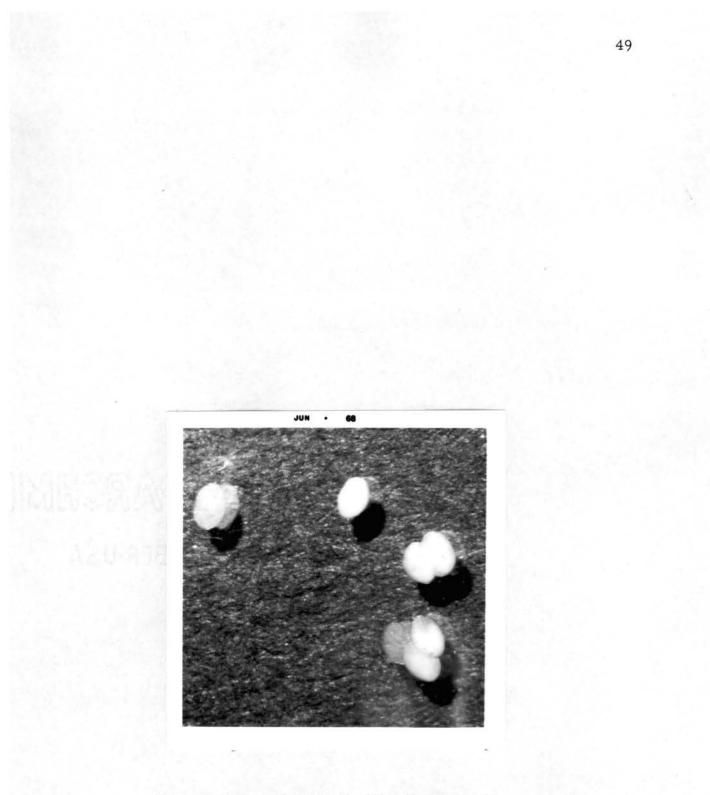


Fig. 5. Newly laid unfertilized eggs of <u>D</u>. unionalis.

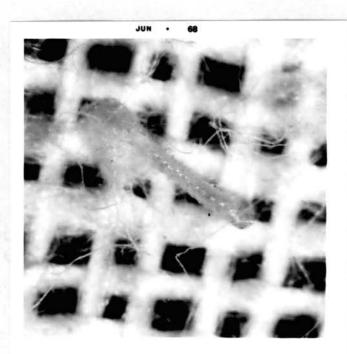


Fig. 6. First larval instar of D. unionalis emerging from the egg.

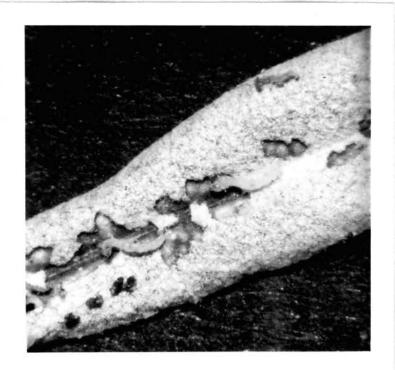


Fig. 7. Feeding of second larval instar of <u>D. unionalis</u>.



# Fig. 8. Feeding of the last larval instar of <u>D.</u> unionalis.



Fig. 9. The prepupal stage of D. unionalis.



Fig. 10. Pupa of <u>D.</u> unionalis.

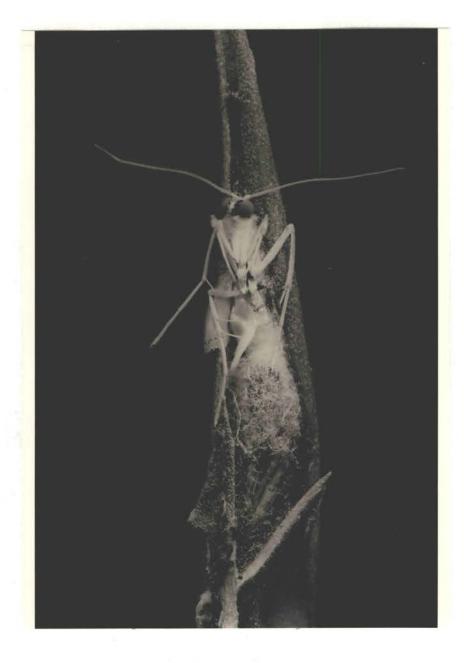


Fig. 11. Emergence of adult <u>D.</u> <u>unionalis</u> from the pupa.



Fig. 12. Infestation of olive flowers by Z. <u>oleastrella</u>.



Fig. 13. Infestation of terminal buds by larvae of  $\underline{D}$ . unionalis.



Fig. 14. Olive fruits infested by D. unionalis.



Fig. 15. Feeding of the larvae of <u>D</u>. <u>unionalis</u> on the olive fruit.



Fig. 16. Adults of <u>D.</u> unionalis, female and male.

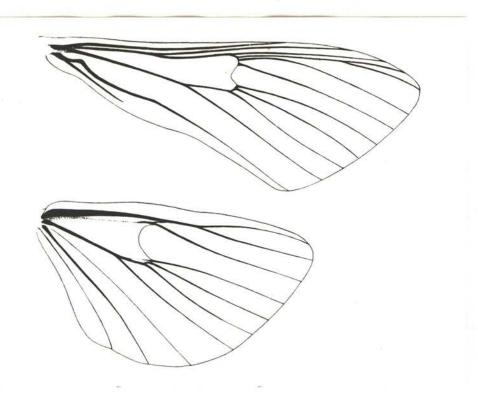


Fig. 17. Wing venation of <u>D.</u> unionalis.

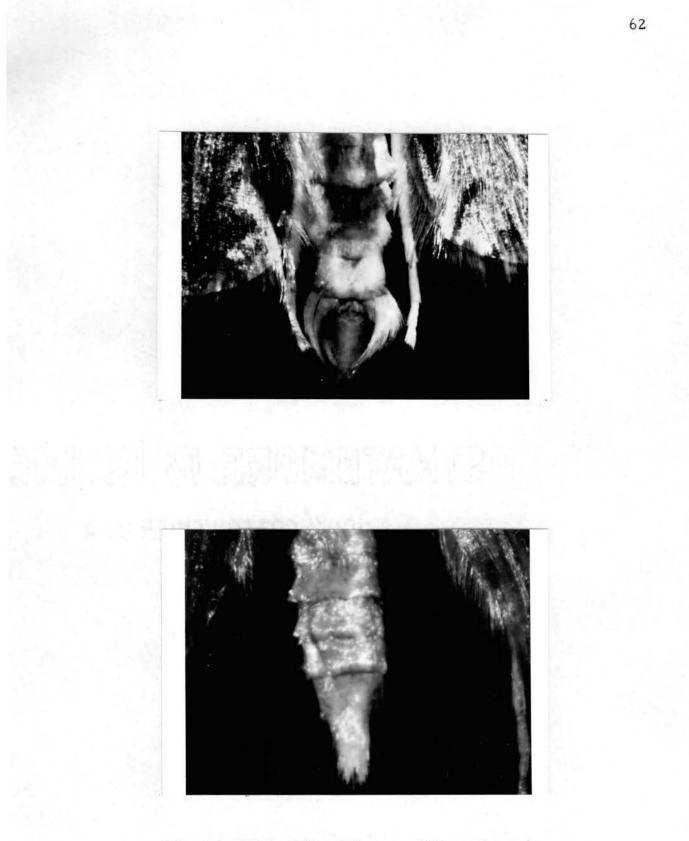
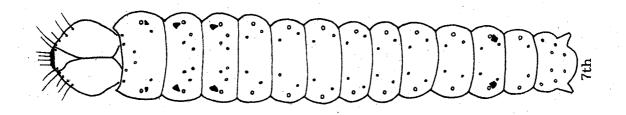


Fig. 18. End of the abdomen of the male and female of <u>D</u>. unionalis.



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3rd 

2nd 

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Fig. 19. Larval instars of D. unionalis.

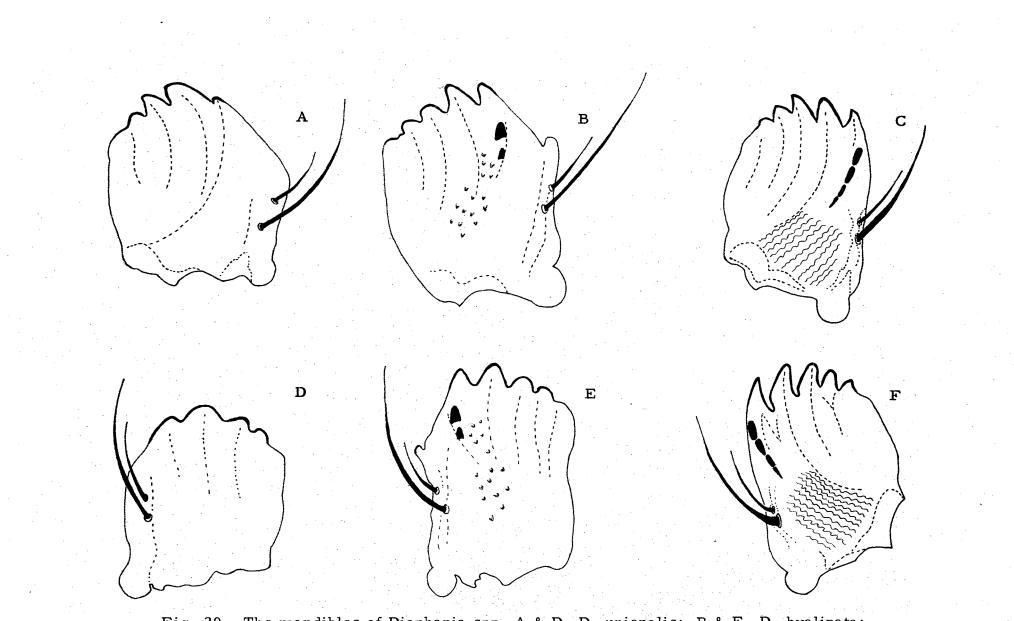


Fig. 20. The mandibles of <u>Diaphania</u> spp. A & D, <u>D. unionalis</u>; B & E, <u>D. hyalinata</u>; C & F, <u>D. nitidalis</u>.

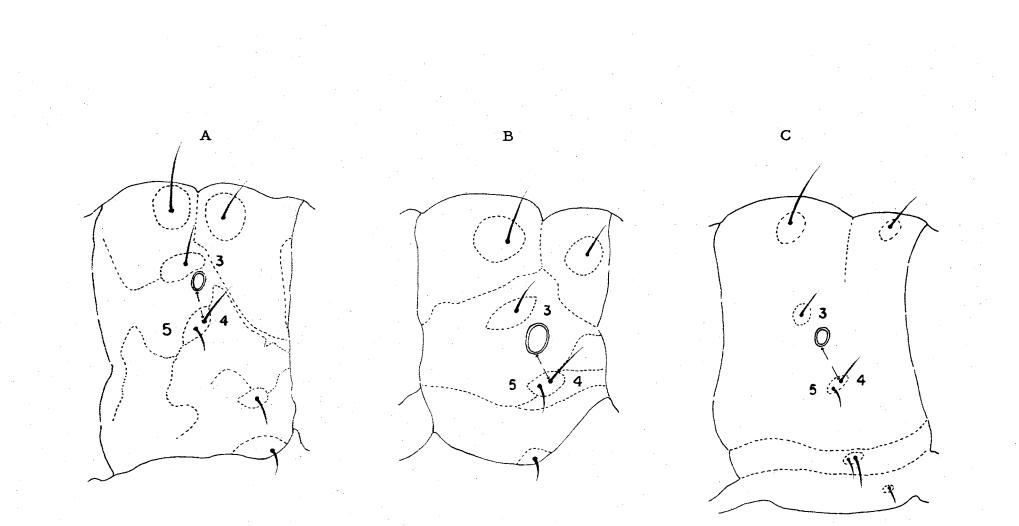
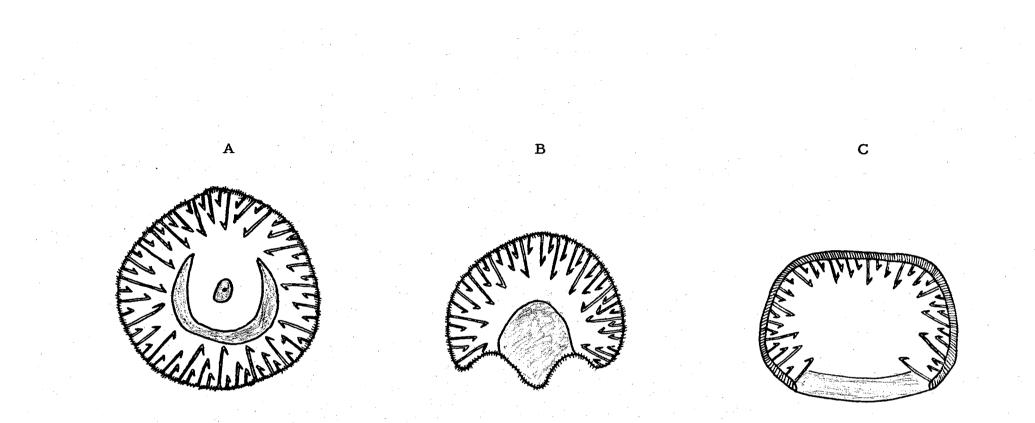
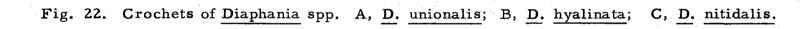


Fig. 21. Eighth abdominal segment of <u>Diaphania</u> spp. A, <u>D. unionalis</u>; B, <u>D. hyalinata</u>; C, <u>D. nitidalis</u>.





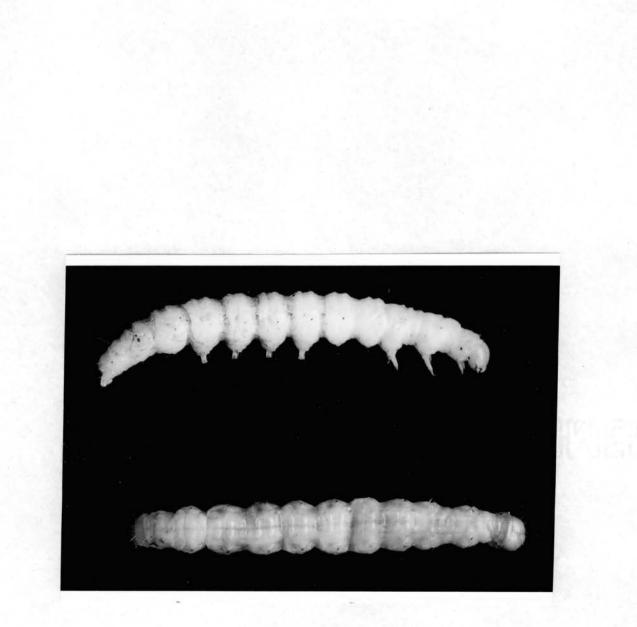


Fig. 23. Full-grown larvae of D. unionalis.

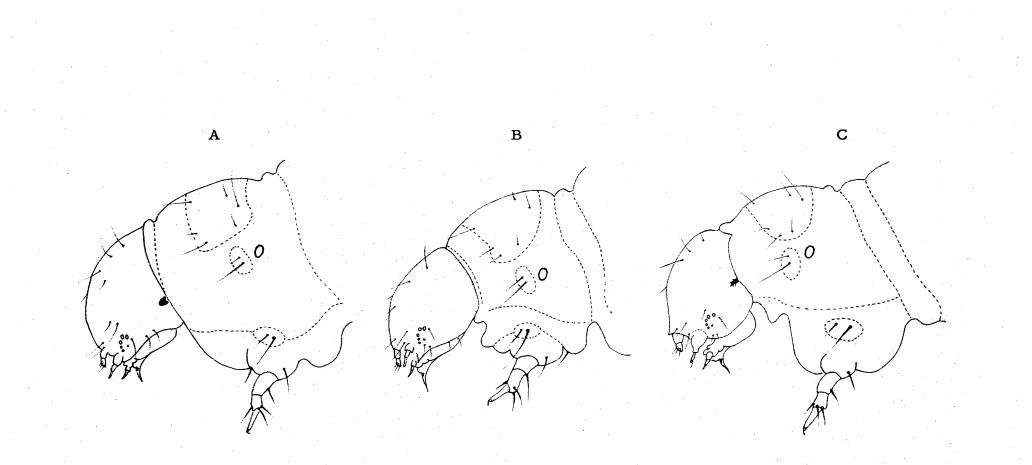
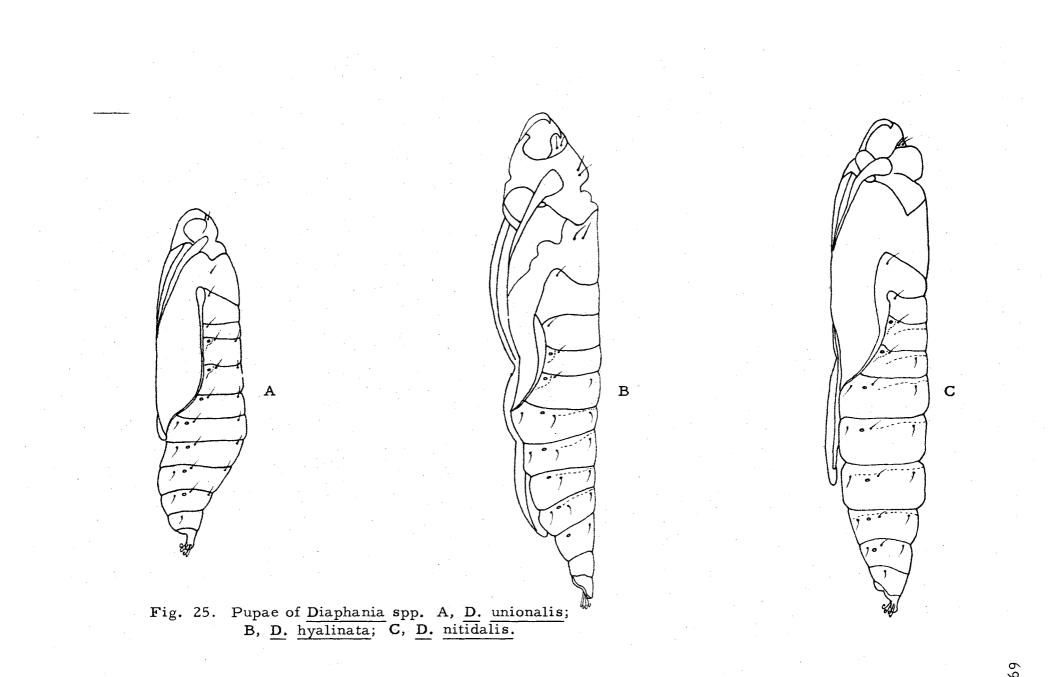


Fig. 24. Anterior regions of Diaphania spp. A, D. unionalis; B, D. hyalinata; C, D. nitidalis.



#### VITA

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Doctor of Philosophy

# Thesis:BIOLOGY AND ECOLOGY OF DIAPHANIA unionalis (Hübner),AND COMPARATIVE MORPHOLOGY OF D. unionalis(Hübner), D. hyalinata (Linnaeus), AND D. nitidalis (Stoll)

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