HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE PUBLISHED BY THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

Volume 35, Number 18 • August, 1964

Mating and Oviposition Behavior of The Navel Orangeworm, *Paramyelois transitella* (Walker)

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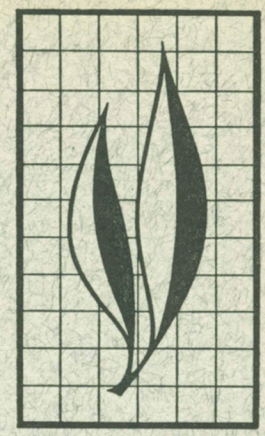

Laboratory studies on the mating behavior of the navel orangeworm, *Paramyelois transitella* (Walker) showed that mating is stimulated by a twilight period. Multiple mating is not uncommon, but males tend to mate more readily with virgin females. Oviposition begins on the second day after mating and reaches a peak on the eighth day.

Studies on black light attraction in the greenhouse showed a diminishing percentage of trapped females in relation to males as the distance from the trap was increased. The heavier bodied females are poorer flyers than the males. This may explain the high ratio of males to females captured in black lights in the field.

Black lights in walnut orchards captured numerous navel orangeworm adults and dissection of the females showed that the majority were in the early to mid stage of reproductive maturity. A method of classifying the females into reproductive age groups was developed in the laboratory using the physiological condition of the females as a criterion. Multiple mating in the field increased as the season progressed and coincided with the periods of highest adult activity.

The data from these studies indicate that black lights are a valuable indicator of navel orangeworm field activity. The mating and oviposition behavior studies provide information that is necessary if a program of control by sterile males is contemplated.

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James A. Goodwin and
Harold F. Madsen

The Mating and Oviposition Behavior of the Navel Orangeworm, *Paramyelois transitella* (Walker)[1]

INTRODUCTION

THE NAVEL ORANGEWORM, *Paramyelois transitella* (Walker), presents a serious and growing problem in northern California. This insect has evolved from a minor pest on oranges to a major pest on nuts. In northern and central California it is particularly damaging when it occurs together with a codling moth infestation. The larva of the navel orangeworm is not able to penetrate the husk of a sound nut and the codling moth damage to the nut provides an entrance route. The increasing number of publications concerning this pest during the past several years reflects the importance of the navel orangeworm to the nut industry. However, as yet, there is no effective method of control for this insect (Wade 1961).

Mating and oviposition studies were made in the laboratory to determine the egg production, mating frequency, and physiological condition of mated females. These data were obtained by using a varying sex ratio and various time periods and ages. The purpose of these tests was to provide a basis for a method of determining reproductive age of mated navel orangeworms in both the laboratory and the field.

A scale of physiological condition in relation to reproduction was set up to determine what specific groups of females were attracted to black light traps. The physiological classification used is similar to the one described by Gehring (1962).

Black light attraction tests were first run in the greenhouse and later limited tests were conducted outside the greenhouse. From these tests the sex ratio and age of the navel orangeworms attracted to black light were determined. Moths of predetermined age were released in the greenhouse, and the percentage trapped was recorded. The results were then related to the catches made from black light traps located at various sites in the field.

The navel orangeworm adults taken from the black light traps in the various field locations were examined and the sex ratio and reproductive age of the females determined. Spermatophore counts were made to determine the number of times each female had mated.

The information gathered from these laboratory and field studies may be of help when control of navel orangeworm through the sterile male release technique is attempted (Husseiny 1962). Data on reproductive age of trapped females coupled with flight records from black light traps may indicate the degree of infestation in a given area as well as the reproductive behavior of females.

Prior to 1920 the navel orangeworm was found along the west coast of Mexico according to Essig (1926). Glick

[1] Submitted for publication February 7, 1964.

(1922) recorded this insect from Guatemala. He also stated that the first specimen taken in the United States was caught in a light trap in Arizona's Salt River Valley on October 4, 1920. However, it had probably been present there since at least the late nineteenth century according to Cockerell (1899). The navel orangeworm spread from Arizona and it was first recorded in southern California in November of 1942 (Armitage 1944). Wade (1961) stated that the moth is a weak flyer and probably does not migrate rapidly by flight. He added that its spread has undoubtedly been aided by transportation in infested materials. Hixon (1934) believed the navel orangeworm had been present in California prior to 1933. Since 1946 this pest has spread northward until it now occurs in practically all the important fruit and nut producing regions in the state (Michelbacher and Ortega 1958).

The navel orangeworm has a wide host range and is able to develop on a variety of hosts, usually when the host has been damaged or becomes mummified. According to Ebeling (1959) the navel orangeworm is of prime economic importance in California to the walnut and almond industries.

Wade (1961) indicated that there was no effective spray program for the control of this pest. Michelbacher and Ross (1955) recommended effective control of codling moth, early harvest, and good sanitation practices as means of reducing the navel orangeworm population.

The navel orangeworm is not attracted to aromatic baits, and another means of trapping field adults was needed. Madsen and Sanborn (1962) found black light was effective attracting the adult moths in the field. They also found the black light traps useful as a means of predicting the degree of navel orangeworm infestation in an orchard. In addition, they reported that black light attracts physiologically young females; but it also attracted a multitude of other night flying insects making separation of the desired species tedious. The seasonal flight pattern of this insect was determined by black light studies by Madsen and Wong (1962). Summers and Marsh (1962) found that the sex ratio of navel orangeworms taken from field black light traps was disproportionate in favor of males. The ratio ranged between 3:1 and 10:1. Because of this ratio, these authors concluded that black light traps may not be an effective agent in the control of the navel orangeworm.

At first the naval orangeworm proved difficult to rear under laboratory conditions. Atkins (1951) stated that the failure to mass produce was usually due to poor mating under laboratory conditions. Wade (1961) encountered difficulty in rearing the navel orangeworm under laboratory conditions. Husseiny (1962) developed a method of mass rearing this moth in the laboratory and thus an adequate number of moths could be readily available for experimental purposes throughout the year. He also concluded, after working on male sterilization of the navel orangeworm, that a program utilizing this technique might prove feasible for field control of this pest.

MATERIALS AND METHODS

Moths used in the laboratory experiments were mass reared in the greenhouse. Most of the original stock of navel orangeworm were obtained from field collections at Walnut Creek (Husseiny 1962). The colony has now been raised under laboratory conditions for about two years. The medium used to rear the moth is the same used for rearing the wax moth *Galleria mellonella* (L.) and consisted of dry pablum 1000 cc, extracted honey 100 cc, glycerine 100 cc, and tap water 50 cc. This amount was sufficient to raise about 1400 moths.

Eighty adult moths were selected randomly from the laboratory population and placed in cylindrical ovipositional cages similar to those used by Sazama (1932). Periodical checks of the sex ratios were made of these randomly selected populations and also the percentage mating was checked. The sex ratio averaged close to 1:1 with slightly more males and the mating ranged between 50 and 90 per cent. The breeding cages were 15 cm in diameter and 25 cm long and were made of celluloid. One end of the cylinder was covered with wax paper and the other with gauze. Both ends were held on by a celluloid ring slipped over the ends of the cylindrical cage. The wax paper end was perforated with small holes made in groups of eight. The gauze end of the cage had a hole 3 cm in diameter through which moths and moisture can be added to the cage. Each cage was provided with a Petri dish containing a cotton pad soaked with water. The interior of the cage was completely lined with wax paper.

The wax paper ends of the cages were then oriented toward a twilight source and during oviposition almost all the eggs were deposited around the holes punched in the wax paper or at the base of the wax paper end. Temperature, air circulation and relative humidity are three important factors in the success of mating, oviposition and egg viability. A fan was used to circulate air through the cylinders. The cages were kept in the greenhouse under a wooden table, and a cardboard partition was located above the table to shield off the radiant heat from the sun. The space under the table was inclosed on three sides by cardboard leaving an open exposure toward the glass wall of the greenhouse. The breeding cages were placed 1 inch above trays filled with water. In addition damp burlap sacks were placed around the edge of the water trays. The action of the fan directed across the inclosed area helped to maintain the temperature and humidity within a favorable range.

When egg production was sufficient, usually by the sixth day, the perforated wax paper end was removed and the egg sheet placed in a glass cylinder 4 inches in diameter and 8 inches long. The bottom end of the glass cylinder was closed with 24 mesh wire screen held tightly in place by a celluloid ring. The cylinder contained the rearing medium previously described to a depth of about 2 inches. The glass cylinders were placed wire end down on a heavy wire screen over a plastic container filled with water to within 2 inches of the bottom of the cylinder. The tray and cylinders were then placed in a sleeve cage similar to that described by Peterson (1959). The sleeve cages were located in a rearing room under continuous artificial light at about 28° C until the larvae began to pupate. The cylinders were then transferred to the sleeve cages in the greenhouse for adult emergence.

The pupae used in the tests were taken from the glass rearing cylinders and were removed from their cocoons by a method similar to that used by Finney *et al.* (1947). The cocoons were placed in a 24 mesh wire basket, about 12 cm in diameter and 20 cm in length. The basket was submerged in a solution consisting of one part commercial bleach containing 5.25 per cent sodium hypochlorite, and two parts water. The basket was kept in the solution for a period of 30 seconds and during this time it was agitated to increase the dissolving of the cocoons. The pupae were then rinsed several times in tap water and placed on paper towels under a lamp to dry. No discernible damage to the pupae was noted during this procedure.

The pupae were separated into groups of male and female. This was done by gonopore determination. On the female pupa the gonopore is found on the ventral side of the eight abdominal segment, and on the ninth abdominal segment in males (figure 1). In the early stage of development the male pupa can be detected by the presence of gonads immediately beneath the dorsal integument

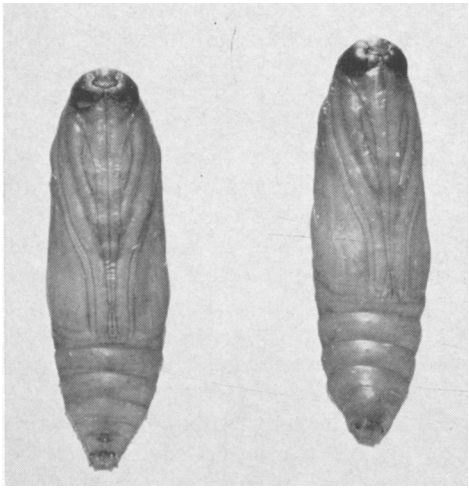

Fig. 1. Navel orangeworm pupae showing the location of the gonopore used in sex determination. *Left:* Male with gonopore located on the sixth ventrally visible abdominal segment. *Right:* Female with gonopore located on the fifth ventrally visible abdominal segment. 6.75 × magnification.

which are visible without dissection. As the pupa matures the integument darkens and the gonads are no longer externally visble. The male gonopore is surrounded by a slightly raised and darker area and is smaller than that of the female. The later instar larvae may be readily sexed with the naked eye. The male gonads are easily visible beneath the dorsal integument (figure 2).

Husseiny (1962) found that pupae could be held in cold storage at 10° C for up to 8 days without any appreciable alteration of fertility, mating or emergence. This technique was adopted in order to have sufficient numbers of sexed pupae emerge at the same time.

The pupae were then placed in a cardboard cylinder with a gauze bottom. This cylinder, measuring 9 cm in diameter, was placed in a sleeve cage located in the greenhouse. The female pupae were placed in one cage and the males in another. In earlier trials it was found that pupae placed in glass vials were subject to higher mortality and many which did emerge were either stuck to the pupal case or had deformed wings. By using the cylinder with a gauze bottom the pupae were able to obtain a purchase for their anal hooks and upon emergence pull themselves free of their pupal cases and avoid wing deformities. A favorable humidity level was maintained by placing these cartons 2 inches above a water level in one-pint Mason jars.

When a sufficient number of adults had emerged in the sleeve cages they were transferred by a suction apparatus to the breeding cages at a predetermined sex ratio. The suction apparatus consisted of a vacuum cleaner modified to provide sufficient suction to remove moths without injury. This apparatus is similar to that used by Dickson *et al.* (1952). At the completion of a test the moths were removed from the cages, anesthetized with CO_2 and stored in 70 per cent alcohol. Later the sex ratio was rechecked and the females dissected.

The adults were separated by the different characteristics of the male and female genitalia. The male genitalia are

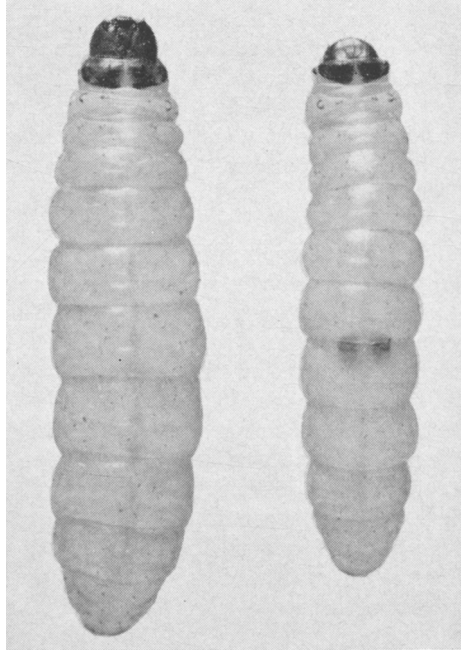

Fig. 2. Navel orangeworm larvae. *Left:* Female. *Right:* Male with externally visible gonads. 6.75 × magnification.

characterized by the presence of claspers and the abdomen has a blunt posterior end. The female is identified by the protruding ovipositor and a rounded posterior tip. Sex identification of the adults can usually be done with the unaided eye. In most cases it is possible to identify the adult males by observing the gonads which are usually visible immediately beneath the dorsal integument of the abdomen. This is especially true when the males have been stored in alcohol for some time and the abdominal scales have been removed.

After the adults were taken from the ovipositional cages the wax paper lining and wax paper ends were removed. The wax paper was cut into smaller sections and the number of eggs recorded. The number of holes in the wax paper ends was constant for all tests. The holes were arranged in two rows of four groups of eight, four rows of five groups of eight, and then two rows of four groups of eight or 288 holes in all. The rows were arranged vertically in relation to the water source under the cages.

The eggs of the navel orangeworm are oval in shape, flattened dorsoventrally and have a reticulated surface. The eggs are shown in figure 3.

Fig. 3. Navel orangeworm eggs. 20 × magnification.

The female moths were taken from the alcohol storage vials and dissected to determine their reproductive condition and the number of matings. The method of dissection was as follows: the abdomen was removed from the thorax and the dorsal integument peeled away. In most cases this procedure exposed the bursa copulatrix if it contained a spermatophore. If no spermatophore was present then it was necessary to probe through some of the fat body tissue and developing eggs to locate the bursa copulatrix. In a few cases the bursa copulatrix does contain a spermatophore which is not immediately visible upon the removal of the dorsal integument of the abdomen. Williams (1941) stated that each spermatophore represents one mating. He also found that some species of Lepidoptera can have more than one spermatophore in the bursa copulatrix at one time. Husseiny (1962) confirmed this in the case of the navel orangeworm and he also concluded that the male passes only one spermatophore per mating.

From the laboratory studies of female moths a system of classifying the females in relation to their reproductive age was developed. This system is similar to that used by Nel (1940) and modified by Gehring (1962). Four groups were set up and any moth considered to be borderline was placed in the next older classification. The four age groupings were as follows:

Group A was made up of females whose abdomens were filled with fat body tissue and predominately immature ova. Females in this group were unmated or those examined one day or less after mating.

Group B consisted of mated females whose fat body tissue was somewhat less than *Group A*. The developing and mature eggs filled most of the abdominal cavity. Moths in this group were females approaching their peak ovipositional period.

Group C comprised moths whose fat body tissue was markedly reduced and

the posterior portion of the abdominal cavity filled with maturing eggs. A vacant cavity in the anterior portion of the abdominal area was present. These moths were usually in the midst of their peak oviposition period. The majority of the moths in the group were from 6 to 10 days old.

Group D consisted of females with very little if any fat body tissue. If eggs were present they were few in number and mature. The anterior body cavity was enlarged. These moths were 10 to 16 days old.

The black light traps used in the greenhouse experiments were of two types. One was a commercial brand called "Luralite" manufactured by the Onamia Manufacturing Company. This trap consisted of a single 30 watt circular U.V. tube mounted to encircle the open end of a sheet metal cylinder. A small fan was mounted toward the rear of the cylinder which provided suction to pull in insects attracted to the light. A screen on the rear end of the cylinder guided the insects into a bag suspended below the trap (figure 4).

The other trap was constructed of galvanized tin and was conical in shape with an upper diameter of 20 inches sloping to a lower diameter of 2 inches over a 23-inch vertical span. The light source was a 15-watt fluorescent black light, 18 inches in length. The tube was set in a fixture which was mounted horizontally across the upper rim of the trap. A Mason jar lid was affixed to the base of the trap so that a quart collecting jar partially filled with alcohol could be attached (figure 5).

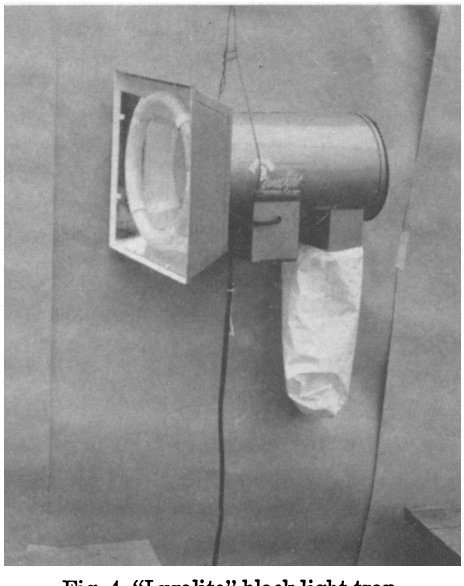

Fig. 4. "Luralite" black light trap.

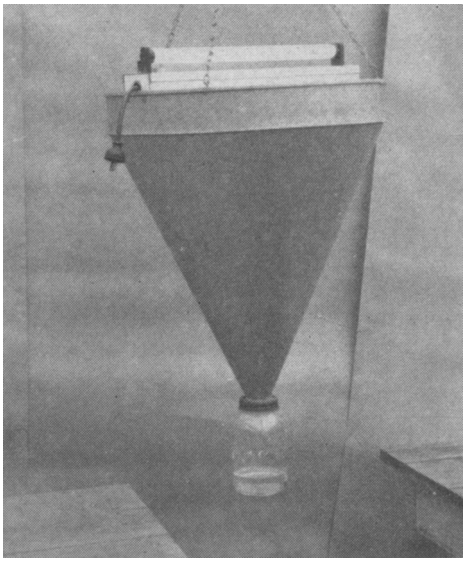

Fig. 5. Conical type black light trap.

These two types of black light traps were tested in several different rooms in the greenhouse to determine how attractive they were to mated and virgin males and females. The data from these tests were compared to determine if one sex was attracted over greater distances and in greater numbers than the other. The catch from these traps was separated by sex and in most cases the females were dissected to determine whether mating and oviposition had occurred.

The galvanized conical type trap was used to obtain most of the specimens in the field. An argon light trap was used to a limited extent in one area. The navel orangeworm catch from the field was divided into male and female groups during the 1962 season. In 1961, a specific record of males was not kept and only the females were retained. The fe-

males taken during both seasons were dissected and spermatophore counts made. Those females caught during the 1962 season were classed according to their reproductive condition.

The laboratory releases were made in two rooms in the greenhouse. The dimensions of the smaller room were 13 feet tall on the high side sloping to 4 feet 6 inches on the low side, 15 feet in width and 20 feet 6 inches in length. The black light trap was placed 5 feet from the floor suspended on a wire strung from the ceiling. In this room the trap was located at the east end of the room 12 feet from the point where the moths were released. The temperature in this room during the tests ranged from 60° to 92° F and the relative humidity varied from 32 to 94 per cent.

The larger room measured 56 feet in length, 18 feet 6 inches in width and 20 feet 6 inches on the high side sloping to 4 feet 6 inches on the low side. The trap was suspended from the ceiling 7 feet above the floor. Releases were made at a distance of 50 feet from the trap. The temperature range in this room during releases was 56° to 91° F and the relative humidity 32 to 94 per cent.

Limited field releases were made outside the greenhouse during favorable weather conditions. These tests were run to determine the distance moths were attracted to the black light and the conical type trap was used. The trap was hung in a tree at a height of 5 feet. The moths were released 110 feet west of the trap.

LABORATORY RESULTS

The mating and ovipositional behavior of the navel orangeworm was determined by laboratory tests run for the following periods: 12 hours without a twilight period, 12 hours with a twilight periods, 1, 2, 4, 6, 8, 10, and 12 days. All of the tests were run on moths immediately after emergence from the pupa. The results from these series are complied in table 1. The most active oviposition period was in the 2- to 10-day range as shown in figure 6. The percentage mated reached nearly 100 after the 4-day test. Multiple mating increased markedly after the second day.

Five females and five males were used per test in the 1- to 12-day series. The results of this series gave a basis for estimating the reproductive age of the females.

The test run with and without a twilight period indicated that the navel orangeworm mating is stimulated by exposure to twilight. In the series with twilight 28 per cent mating occurred, while in the series without a twilight period no mating was detected. A trial was run holding the moths in complete darkness for the 12-hour period. In these tests 12 per cent mating took place. This exceeds the mating recorded from the test run in constant light but is less than half that obtained with a twilight period.

A series of experiments was conducted in an attempt to show the daily egg production for the same female over an entire reproductive life span. Each morning the moths were transferred to a new oviposition cage and the eggs deposited the previous night and day recorded. This series was abandoned due to poor mating, low egg production, and high adult mortality. These results were probably due to the excessive handling required to make daily cage changes. In place of this series, average egg production was taken from tests run over a period of 16 days. Separate tests were concluded at two-day intervals and the number of eggs recorded. By this technique frequent cage changes were avoided. These results are illustrated graphically in figure 7. The graph shows that the maximum egg production is between the 2-8-day periods with a marked drop in egg production after the tenth day. There seems to be a preoviposi-

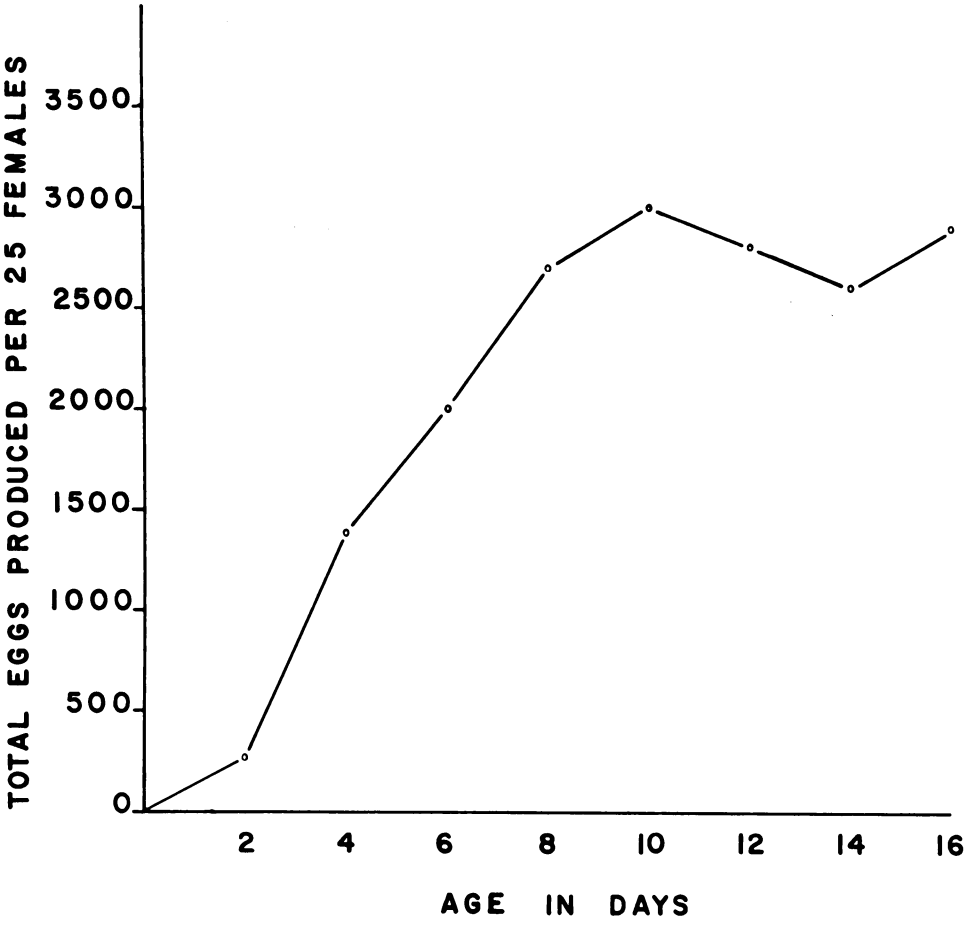

Fig. 6. The total eggs produced per day for a period of 16 days for 25 mated navel orangeworm adults.

tional period of 1 day although virgin females will deposit a few eggs.

Navel orangeworm adults were greatly influenced by changes in temperature, light and humidity during mating and oviposition. The greenhouse did not provide optimum and stable conditions for breeding and ovipositional studies of the navel orangeworm. It was found that differences in mating and oviposition occurred in the area used for the experiments depending upon where the cages were placed with respect to light, moisture and wind source. In many cases the distance involved in the position changes was only a matter of inches. Because of this variability the test cages had to be placed in comparable locations. The temperature outside the breeding cages beneath the table ranged from 52° to 80° F and 60 to 84 relative humidity. The greenhouse, as a whole, had greater fluctuations in temperature and humidity than that recorded in the breeding area beneath the table where an attempt was made to modify the environment. Although conditions were not optimum the data are sufficient to indicate trends at the different ages and sex combinations.

Another series of tests was run for 10 days with a varying sex ratio (table 2). These tests indicate that the male tends to mate with unmated females. In the tests run with fewer females than males there were less spermatophores pro-

TABLE 1

THE OVIPOSITION AND MATING OF NAVEL ORANGEWORM FOLLOWING THE CONFINEMENT OF FIVE MALES AND FIVE FEMALES

	Test period 12 hours				The number of eggs and spermatophores after													
	Without twilight		With twilight		1 day		2 day		4 day		6 day		8 day		10 day		12 day	
	Eggs	Sperm.	Eggs	Sperm.	Eggs	Sperm.	Eggs	Sperm.	Eggs	Sperm.	Eggs	Sperm.	Eggs	Sperm.	Eggs	Sperm.	Eggs	Sperm.
Test 1	0	0	9	2	17	2	62	2	225	5	448	5	632	8	549	5	566	4
Test 2	0	0	0	1	5	2	60	2	188	4	351	7	574	6	595	8	668	6
Test 3	0	0	5	0	9	1	41	3	268	4	449	6	489	4	682	4	554	5
Test 4	0	0	11	3	10	0	50	4	321	7	387	6	436	4	602	8	491	7
Test 5	0	0	13	1	42	1	56	5	293	5	368	7	577	5	581	10	539	7
Total	0	0	38	7	83	6	269	16	1295	25	2003	31	2708	27	3009	35	2818	29

duced per male. A factor which should be considered is the disparity in size of spermatophores produced. This is probably correlated with the size and vigor of the male producing the spermatophore. The adults showed a variation in size, probably due to overcrowding in some of the larval-rearing cylinders. At times the bursa copulatrix of a mated female was filled to the bursting point by one or two large spermatophores and it appeared to be a physical impossibility for a male to insert another spermatophore without either bursting the bursa or collapsing the spermatophores already present. In tests where a single male was confined with five females an average of 1.80 spermatophores was produced per male. In the case of a single female with five males an average of .44 spermatophores was produced per male. The egg production in tests containing five females and one male was below that obtained in earlier tests. This was probably due to both the time required for the male to form the spermatophore and copulate with the female and the reduction in egg production because of unmated females. Several of the females in the tests were probably not fertilized until the test was almost over. Tests run with caged virgin females indicated that unmated females will oviposit but their total egg production is only 16 per cent of the average mated female over a 6-day period.

A series of tests was run to determine if prior egg deposition was a stimulus for females to oviposit. Groups of eight holes in the wax paper ends of the breeding cages were selected at random. These areas were painted with a solution of crushed eggs of various ages in distilled water. After several groups of eight holes were treated 80 male and female adults were added to the cage. After 8 days the adults were removed and the number of eggs counted in both the treated and untreated areas. An untreated check cage was included during the same time and the egg dispersion compared with that of the treated cage.

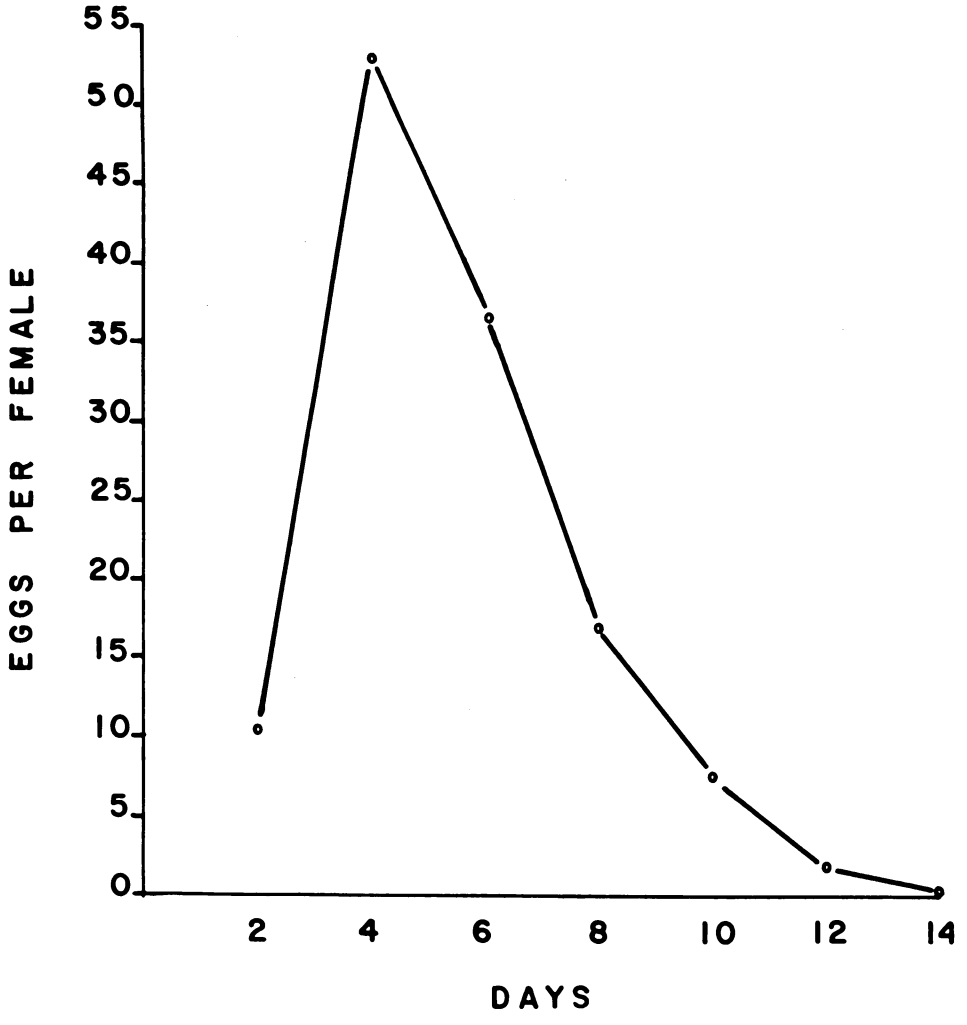

Fig. 7. The egg production per female navel orangeworm over a span of 14 days.

TABLE 2

THE EFFECTS OF DIFFERENT SEX RATIOS ON THE MATING OF THE NAVEL ORANGEWORM OVER AN 8-DAY PERIOD

	Number of spermatophores produced with		
	5 male: 1 female	5 male: 5 female	1 male: 5 female
	2	5	3
	3	7	2
	2	4	1
	3	8	2
	2	7	1
Total	12	31	9
Mean spermatophores produced per male	.44	1.24	1.80

The treated areas showed an increase in egg deposition over the untreated areas. The proportion favoring the treated areas increased when the areas were located nearer the bottom of the cage. The increase over the untreated areas ranged from 52 per cent to 138 per cent. The total egg production in the treated cages was slightly higher than that in the untreated check cages. The general trend in the breeding cages is toward higher egg deposition on the lower areas of the wax paper end of the breeding cage. This may be due to the confinement of moths in a small area. As the females became older and weaker they no longer climb any distance up the cage to deposit their eggs.

During the mass rearing program a darker colored navel orangeworm adult was found. Several of these dark moths were placed together in an attempt to isolate this strain. Although this one effort was not successful it should not be difficult to isolate this strain. If successful, such a color variant could be useful for field releases nullifying the need for artificial tagging. Further tests would be necessary to determine normal behavior and competitiveness of this darker moth as compared to the wild population.

RESULTS WITH LABORATORY BLACK LIGHT

The two traps used were similar in their attractiveness to the navel orangeworm adults. The conical type used in the field proved to be slightly better than the fan type trap in attracting moths. The "Luralite" trap, because the moths were pulled through the fan, mutilated some of the trapped moths and sex determination and age classification was impossible. This reason in addition to the "Luralite" trap being slightly inferior as an attractant led to the use of the galvanized conical trap in all tests except those where only one sex was involved. Both traps were not capable of capturing all moths attracted to them. Therefore, the moths found sitting on each trap in the morning were included in the previous night's catch.

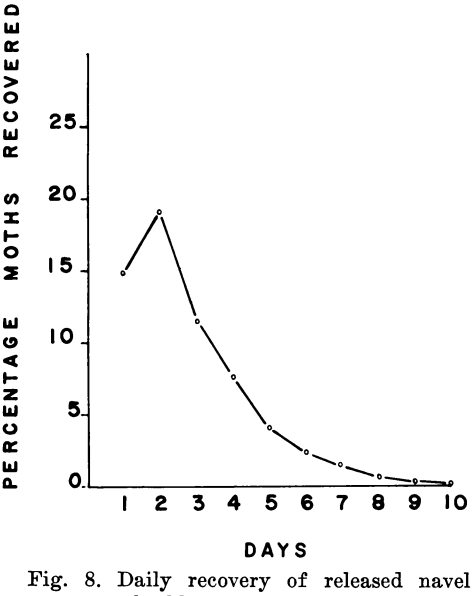

Fig. 8. Daily recovery of released navel orangeworm by black light traps in the greenhouse.

The first series of tests were designed to determine the percentage of released moths attracted to the black light over a period of time. Two thousand moths were released in groups of 100 to 400 over a period of several months. These releases were made in the smaller room in the greenhouse. The moths varied in age at time of release from 0 to 2 days. The results of these tests are shown in figure 8. The greatest number of the moths trapped were caught within five nights after release. The moths found dead at the release site were deleted from the total released. An over-all percentage of 61.8 of the released moths was recovered. These moths were sexed and the females dissected to determine the number of matings. The sex ratio of the recovered moths showed a ratio of 1:1.8 in favor of the male. The females comprised 35.7 per cent of the catch and the males 64.3 per cent.

TABLE 3

THE ATTRACTIVITY OF BLACK LIGHT FOR MATED AND UNMATED MALES AND FEMALES RELEASED IN THE GREENHOUSE OVER A 10-DAY PERIOD

	Unmated males		Unmated females		Mated males		Mated females	
	Number released	Percent captured	Number released	Percent captured	Number released	Percent captured	Number released	Percent captured
Test 1	91	72.1	73	45.2	45	62.2	33	15.2
Test 2	70	64.3	87	40.2	42	71.7	45	28.9
Test 3	99	58.5	98	46.9	42	64.3	41	21.9
Mean percentage	..	64.8	..	44.1	..	63.1	..	22.0

The next series of releases was made in the larger room in the greenhouse. Equal numbers of males and females were released in the evening and collections were recorded the following morning. The results showed an increase in the number of males over females recovered at the 50-foot distance. A total of 293 males was released and 113 recaptured for a recovery percentage of 38.6 per cent. Of the 284 females released 47 were recovered for a percentage of 16.2. The females comprised 29.4 per cent of recovered moths and the males 70.6 per cent. The sex ratio was 2.4:1 male to female. The sex ratio of these moths at time of release was nearly even being 1.03:1 in favor of the males.

Several releases were made outside the greenhouse at a distance of 110 feet west of the black light trap. These tests were limited because of adverse weather conditions. A total of six hundred moths was released over a period of several weeks in late September and early October of 1962. The total catch for these tests amounted to eleven males and one female. The weather during these tests was cold and therefore not optimum for navel orangeworm flight or mating.

A number of releases consisting of males was made in the smaller room in the greenhouse followed by releases of virgin females. The data obtained from these tests are compared and shown in table 3. Mated males and females were also released and compared with the virgin males and females. The mated moths were kept in breeding cages at a known sex ratio for three days prior to release. The females captured were dissected to determine if mating had taken place. Females that were unmated were not included in the totals. The data in table 3 show an increase in capture of unmated females over the mated females. Black light may be more attractive to unmated females or the decrease in mated females may be due to the older age chronologically at which they were released.

FIELD RESULTS

Black light traps were in operation in several walnut, pear and almond orchards throughout most of the 1961 and 1962 seasons. These traps were located at the Experiment Station at San Jose, the Perry orchard at Walnut Creek, the Anderson orchard at Linden and the Ungerman orchard at Live Oak. The males from the catches made during the 1961 season were discarded before an accurate sex ratio for the year's catch could be made. The sex ratio obtained from black light traps as the 1962 season progressed is shown in figure 9. The total number of navel orangeworm adults examined from the field in 1962 was 1410. The sex ratio was 5.13:1 male to female. The number of females who were multiple mated showed an increase as the season progressed (figure 9). A total of 94.4 per cent of the females was mated and of these, 16.5 per cent had mated more than once (table 4). A Chi-Square test for independence showed a

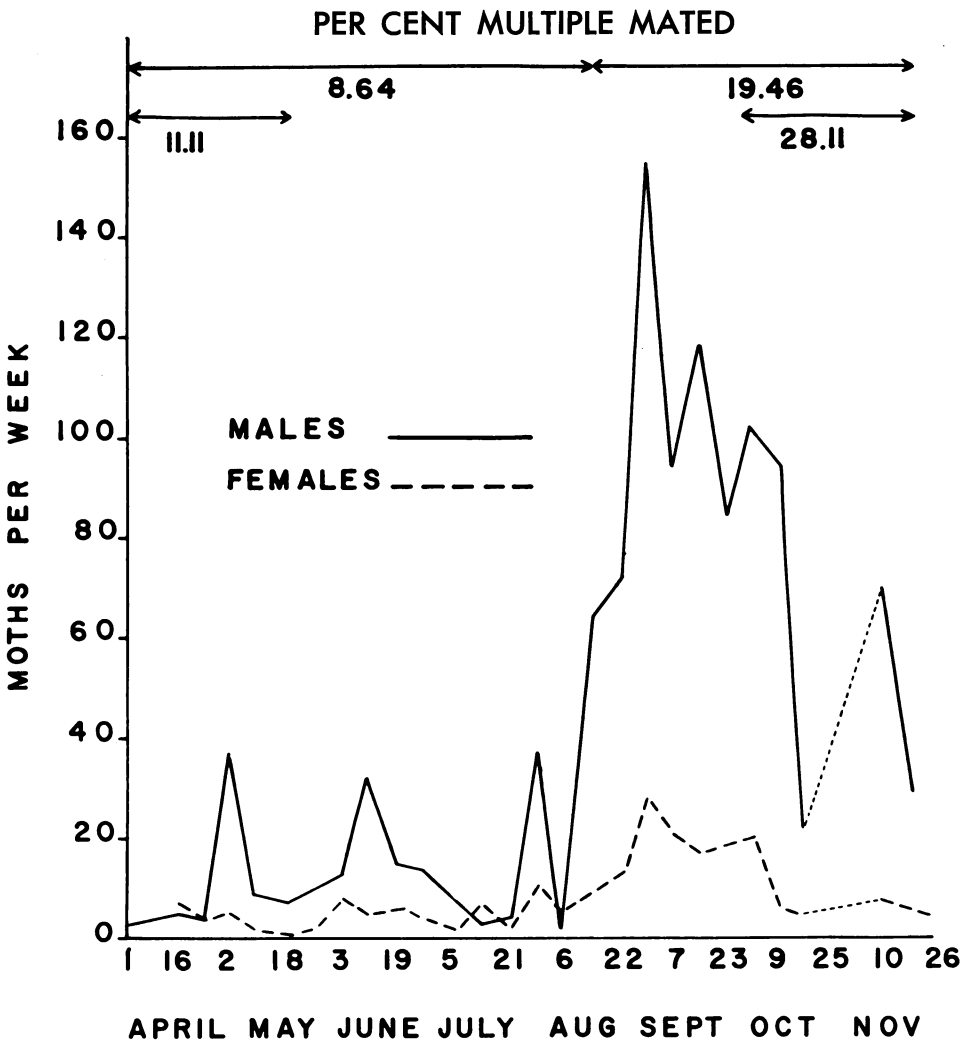

Fig. 9. Navel orangeworm catch in black light traps located in the field during 1962. Small dotted line indicates a period when the catch was lost.

TABLE 4

THE INCREASE IN MULTIPLE MATED FEMALE NAVEL ORANGEWORM IN THE FIELD AS THE SEASON PROGRESSED IN 1962

Date	Number of recovered—		Number of females with spermatophore numbers of—				Percentage multiple
	Male	Female	0	1	2	3	Mated
4-1/5-15.....	56	18	2	14	2	0	11.1
5-16/7-1.....	88	22	1	20	1	0	4.5
7-2/8-15.....	143	41	1	36	4	0	9.7
8-16/10-1.....	679	117	2	95	19	1	17.1
10-2/11-15.....	214	32	6	17	8	1	28.1
Mean.....	...	...	..	..	..	..	14.1

Chi-Square test P — 0.10

TABLE 5

THE REPRODUCTUVE AGE GROUPS OF ADULT FEMALE NAVEL ORANGEWORM CAPTURED IN BLACK LIGHT TRAPS

Date	Number of females	Percentage of total			
		Group A	Group B	Group C	Group D
4-1/5-15	54	11.1	37.1	44.4	7.4
5-16/7-1	76	7.9	26.3	50.0	15.8
7-2/8-15	46	4.4	30.4	56.5	8.7
8-16/10-1	262	5.4	39.7	37.0	17.9
10-2/11-15	248	8.9	27.4	41.1	22.6
Total	686	Ave. 7.3	32.8	41.9	17.9

L S D.05 — 9.5
L S D.01 — 13.3

P value of approximately 0.10. The period from October 2 to November 15 provided 54 per cent of the Chi-Square total. The increase in multiple matings coincides with the periods of heaviest flight activity as shown by Madsen and Wong (1962) in their studies of the navel orangeworm flight patterns. Adding the female data from the 1961 season to those of the 1962 season gave a 92.3 per cent incidence of mating and 13.9 per cent frequency of multiple mating. In the laboratory the incidence of multiple mating in tests over 4 days' duration was 30.6 per cent. This difference was probably due to the confined area of the breeding cages.

The females taken in 1962 were classified as to reproductive age by the grouping previously described. The data show that the majority of females attracted to black light are in Group B or Group C. Very few females captured were in the reproductively immature Group A or in the reproductively spent Group D (table 5). This indicates the black light does not tend to attract the very young or the very old females. Most of the females captured are in the early and middle stages of egg production and are still quite active as far as oviposition is concerned.

In one area in the field an argon light trap was used. This trap was the same as the conical type trap except that the black light tube was replaced with a 4-watt argon bulb. The catches from this trap were not as numerous as those from a black light trap in the immediate vicinity. The argon trap did attract fewer species of night-flying insects and thus reduced the sorting required to detect the catch of navel orangeworm.

SUMMARY AND DISCUSSION

The laboratory studies indicate that the navel orangeworm is stimulated to mate by a twilight period. Mating does occur on the first night after emergence but does not reach the 90 to 100 per cent level until the fourth night. Mating was severely curtailed under conditions where a twilight period was absent.

In laboratory tests it was shown that a navel orangeworm male is capable of mating more than once. When a single male was placed with five females the average number of spermatophores produced was 1.80. When five males were caged with one female the average number of spermatophores produced was .44. This indicates that the males tend to mate with virgin females rather than those females already mated. The size of the first spermatophore inserted may be an obstacle to immediate insertion of another. However, there may be some stimulus produced by the unmated female which induces the male to seek out

virgin females. Cages containing five males and five females produced an average of 1.24 spermatophores per male. In the field only three females were found with a total of three spermatophores. In the laboratory a few were found with four and one was found with five. The close confinement in the breeding cage is probably the main cause of the higher incidence of multiple mating in the laboratory. The variation in size of the spermatophores produced by males reared in the laboratory may also be a factor. Figure 10 shows some of the variation in spermatophore size. In general, spermatophores from field specimens were more uniform in size. The males in the greenhouse produced some small spermatophores, several of which could easily be accommodated in the bursa copulatrix of the female.

The females have a preovipositional period of one day. On occasion, unmated females will deposit a few eggs. The site of ovipositing seems to be enhanced by the presence of a mixture of crushed navel orangeworm eggs and distilled water. The peak oviposition occurs by the eight day and the moths are reproductively spent after the twelfth day. The maximum egg production may, in the case of the laboratory females, be delayed over that occurring in a wild population. This may be due to handling and close confinement. Wade (1961) found that if egg deposition is slow then the duration of the egg-laying period is extended. If a premating flight is necessary, the close confinement and observed domesticity of the laboratory colony could explain a delay in oviposition. A larger breeding cage might be an aid to more rapid mating. The unstable weather conditions in the greenhouse were also an important factor in the variability observed in both mating and oviposition. The greatest single day's egg production occurred between the third and sixth day with a rapid decline thereafter. Mating in the breeding

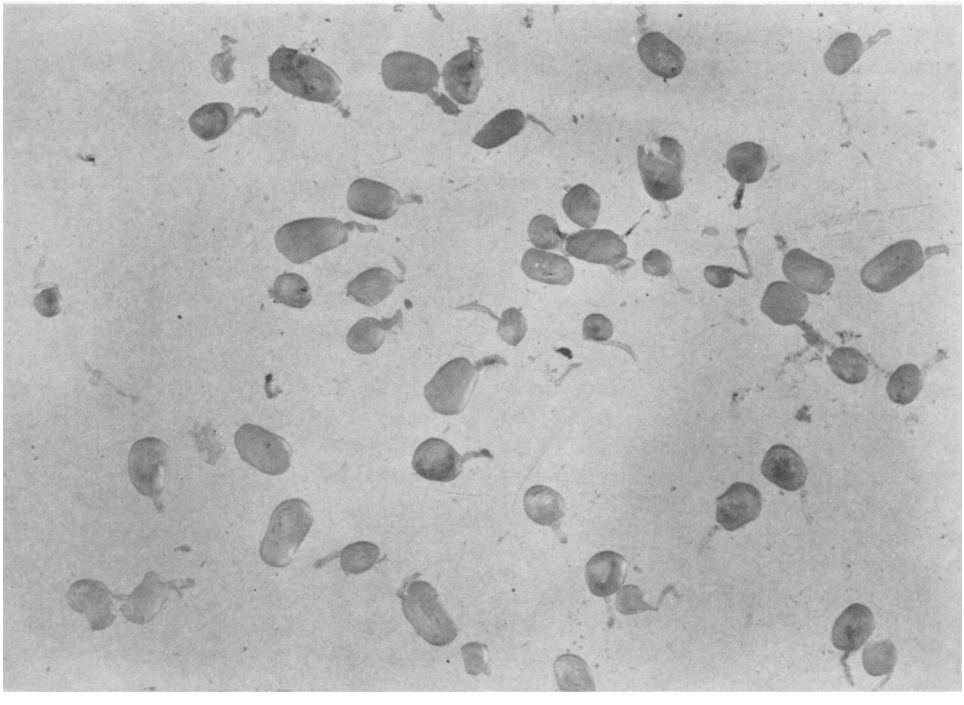

Fig. 10. Spermatophores from navel orangeworm showing the size variations among the individual spermatophores. 6.25 × magnification.

cages reached 60 per cent by the second day and 90 to 100 per cent by the fourth day. Multiple mating increased from the fourth day until the tenth day. Some delay in oviposition might be caused by mating activities in the breeding cages. Wade (1961) found that copulation took between 2 and 5 hours and in one instance 9 hours.

The sex ratio obtained from the black light field catches was 5.13:1 males to females. An examination of the sex ratio of the moths used in the laboratory showed a ratio of 1.15:1 males to females. Moths reared from field larval catches were close to 1:1. This indicates that the sex ratio of moths attracted to black light traps is not representative of the navel orangeworm sex ratio in the field.

Black light studies conducted in the greenhouse and to a limited extent outside the greenhouse showed a diminishing percentage of trapped females in relation to males as the distance from the trap is increased. The heavier bodied females were poorer fliers than the males. This fact together with a slight natural numerical superiority of males partially explained the uneven sex ratio obtained from field catches. The search for an egg-laying site and the act of oviposition may reduce the number of females susceptible to black light. The ability of black light to attract more males than females may be influenced by these ovipositional needs of the females. The over-all catch in the laboratory showed the largest return during the first four nights after release. Considering age at time of release these moths were from 3 to 6 days old. The catch made in the small greenhouse room showed a 61.8 per cent capture over a 10-day period. The inability of the black light to attract a higher percentage of those released in such a small area combined with the uneven sex ratio obtained in the field indicated that black light traps would not be a successful method of control. A factor which may have reduced the over-all catch in the greenhouse was the reflection of the black light off the glass walls and ceiling of the greenhouse room. This may have a confusing effect on moths causing them to fly to the end of the room where the trap was located but not necessarily to the trap itself.

A count of spermatophores from the bursa copulatrix of the females taken from the field revealed an increase in multiple mating as the season progressed. This increase tended to coincide with the periods of greatest flight activity. The higher population levels during these periods explain part of the increase in mating. Weather conditions may also be responsible for some of the mating variation. The laboratory breeding experiments revealed the importance of small differences in temperature and humidity which can have an important effect on mating and oviposition.

The percentage of multiple-mated females in a population is very important when sterile male release is being considered as a method of control. The optimum condition would be the case where the females mate but a single time. Results from the spermatophore checks of females from the field show 12.8 per cent had mated more than once and 0.5 per cent had mated more than twice. Husseiny (1962) concluded that a sterile male control program for navel orangeworm would be feasible. The ratio between normal males and released sterile males must be in favor of a higher number of sterile males to be successful in the control program. A black light could be used to attract the normal moths in an area immediately prior to release of sterile males. By proper coordination with flight data the lights could be used at the beginning of a brood flight to reduce the male population. Since the percentage of multiple matings increased in the late season, it would be preferable to release sterile males early in the season, when the overwintering brood emerges. The competition for the female would then be reduced and the chances of sterile males mating with virgin or once mated fe-

males would be increased. Black light could also aid in determination of field population. Coupled with damage data the black light catches could give an indication of the success of a sterile male control program.

A grouping using physiological condition of the female moths was developed to classify the reproductive age of the females caught in the field. This grouping was based upon the amount of fat body and maturity of eggs in the female abdomen. It was found that the majority of the females attracted to the black light traps were in early or middle stages of their reproductive maturity. Group B and Group C comprised 74.7 per cent of the total female catch. The moths in these classes are approaching or are in prime reproductive condition. Group A made up 7.3 per cent of the total and consisted of unmated and newly mated females. Group D comprised 18.0 per cent of the catch and contained those moths considered reproductively spent. The large percentage of females attracted early or midway in their reproductive spans attests to the value of the black light trap as an early indicator of navel orangeworm field activity.

ACKNOWLEDGMENTS

The assistance of Professors A. H. Gold and T. E. Mittler during the course of this investigation is gratefully acknowledged. Dr. M. M. Husseiny contributed specimens for the establishment of the navel orangeworm colony and made a number of valuable suggestions which aided the study. Thanks are also due to the Onamia Manufacturing Company for the commercial black light trap used in the attraction studies.

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4*m*-8,'64(E6102)J.F.

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