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Larval Development and Timeliness of Pupation in the Laboratory of the Navel Orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Phycitidae), on Certain Diets, under Various Photoperiod, Temperature, Aeration and Humidity Conditions[1]

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ABSTRACT

Development of larvae of the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Phycitidae), was studied under various photoperiods, temperatures and larval diets, in an effort to induce dormancy. Ready - to - hatch eggs or neonate larvae were placed in transparent vials half - full of diet. Fully grown larvae not pupating within 14 days at 26.7°C and L:D 16:8 were considered as being in dormancy.

With artificial larval diets containing bran, yeast, vitamins, and fortified or not with a high protein cereal and egg yolk, none of the treatments induced dormancy to a substantial percentage of laboratory stock or wild larvae. With dry walnut meats, larval growth was slower and survival much lower than with the artificial diets. With walnut meats, when eggs were incubated at 32.2° and L:D 0:24, a certain percentage of grown larvae of the laboratory stock underwent dormancy when grown as larvae under the conditions that follow: 40% at 21° and L:D 12:12, 17% at 21° and L:D 0:24, 19% at 26.7° and L:D 16:8, and 22% at 26.7° and L:D 0:24 for the first 14 days than at 21° and L:D 8:16 for the rest of larval life. When both embrya and larvae developed at 21° and L:D 0:24, 17% of the larvae underwent dormancy. Yet, the relatively small number of grown larvae in the groups fed walnut meats suggests further work for the occurrence of dormancy in this insect to be proven.

No larvae developed on straight brewers' yeast powder. Straight soybean flour or 9:1 and 7:3 mixtures of it with yeast powder allowed the production of grown larvae, pupae and adults of normal appearance. The rate of larval growth on the soy:yeast diets was significantly slower than on a reference diet.

At 26.7°C, a L:D 16:8 photoperiod was as good as a 14:10 one. Continuous darkness resulted in significantly reduced yield in adults and rate of larval growth.

High relative humidity on the surface of the diet allowed excessive growth of fungi on and in the soy:yeast diets and resulted in a much faster larval growth.

Larvae developed well on dry walnut meats and in cracked dry and water-soaked walnuts. Inside the walnuts the rate of larval growth was uneven, some larvae being still fairly small on the 53rd day at 26.7°, while the majority was fully grown or had already pupated.

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Introduction

The navel orangeworm (NOW), *Amyelois transitella* (Walker), while being primarily a scavenger, is a serious pest of almonds, walnuts and pistachio nuts in California (Ebeling 1959, Michelbacher and Davis 1961, Rice 1978). In almond, walnut and orange orchards it overwinters as larvae in various instars in the mummy fruits which remain on the trees, or have fallen to the ground (Glick 1922, Michelbacher 1958, Wade 1961, Falcon 1964). The larvae continue their development during sufficiently warm winter periods and complete their development in late winter and early spring (Michelbacher and Davis 1961, Caltagirone et al. 1968, Engle 1981, Sanderson 1986). In spring, emerging moths oviposit on mummy nuts and the larvae of the first generation and a percentage of the second generation develop in such mummy nuts. In summer, when the new crop fruit start to dehisce, oviposition turns mainly to such fruit (Caltagirone et al. 1968, Rice 1976, Engle 1981).

According to the literature, this insect does not spend a certain season of the year at a specific stage of development as would be the case if its populations annually enter a dormant stage. Furthermore, various larval instars collected in winter and placed under higher temperature conditions, developed immediately to the next stage (Wade 1961). Thus, a number of investigators conclude that the NOW has no diapause in California (Michelbacher and Davis 1961, Wade 1961, Summers 1962), and others doubt the occurrence of diapause in this insect (Sanderson 1986).

On the other hand, Gal (1978) reported that, for the first time, he observed diapause in the NOW and that a photophase below 10 hours is important. Unfortunately, he gave no further details concerning the stage during which the short photophase was important, the stage in which diapause was manifested, or his criterion of diapause. Legner (1983) observed that a certain percentage of navel orangeworms he field collected in August and December in northern, central and southern California developed to the adult stage, at 25.6°C and L:D 14:10, much later than individuals of a laboratory stock, and concluded that this indicates diapause. Furthermore, there are observations of some investigators which may indicate that, under certain conditions, some larvae of the NOW behaved differently from the rest, in a way which reminds one of predormancy behavior in some other Lepidoptera. According to Glick (1922), "cocoons found in hard dried oranges on the ground (in Arizona) may lack the elongation". He refers to the elongation of the cocoon forming a tube allowing the emergence of the adult moth. Cocoons found by Glick on the outside of oranges also lacked the elongation, while in vials the elongation was retained. Glick further reported that the winter cocoons are heavy and coarse, while those found during the summer and early fall, especially in the oranges on the trees, are very flimsy and the pupa may be easily seen through the silk. Fully grown larvae removed from hibernation in dried oranges usually pupated within 2 or 3 days. Those larvae invariably spun their cocoons, whether they pupated soon after or not. Sanderson (1986) had inconsistent results in a study of developmental rates from neonate larva to adult, at various temperatures at L:D 14:10, because of abnormal behavior of last instar larvae. Those larvae, which grew inside mummified almonds, on full growth abandoned the almonds and remained in the last instar for an unusually long period of time, spinning extensive emergence tunnels and/or several partially completed cocoons. They did not feed, their size decreased with time, and some pupated without adults emerging.

Given the opposing views in the literature, we felt that it was worth while to obtain experimental evidence to clarify the matter, by submitting navel orangeworms to various photoperiod end temperature conditions. Development on a few simple diets under various illumination, aeration and humidity conditions was also tried, to possibly detect delays in development which might indicate a tendency towards dormancy.

Materials and Methods

Timeliness of pupation

Laboratory stock. The insects were of a laboratory colony, originating in southern California and maintained for approximately 25 generations in a room of 25.5°C receiving natural daylight through a window. The larvae had been reared on an artificial diet (A below), which is a modification of the diet used by Finney and Brinkman (1967). Most of our

experimental larvae were reared on the same diet and some on a diet fortified with more yeast, a high protein cereal, and hen's egg yolk (Diet B below). The purpose of having two artificial diets in one of the experiments was to increase the chances of dormancy, in view of the fact that in some other insects dormancy induction was affected by certain components of the larval diet (Beck 1968, Tauber et al. 1986). Larvae were also reared on walnut meats taken out of entire nuts purchased in April 1986 from a local grocery. The walnut meats were broken into pieces 2 to 10 mm in size.

	Diet A	Diet B
Wheat bran, unpacked	1200	1350 ml
Gerber high protein cereal	–	113 g
Brewer's yeast powder (NBCo Biochemicals, Cleveland, Ohio)	5	45 g
Vitamin mixture	6	6 g
Hen's egg yolk, fresh	–	20 g
Honey	100	133 ml
Glycerin	100	133 ml
Tetracycline hydrochloride	83	83 mg
Water, distilled	50	66 ml

From March 10 to April 6, 1986, adults emerging from pupae were maintained at 20°C in a laboratory room receiving natural daylight from a northern window, in glass battery jars covered with white tissue paper. The eggs were laid on the tissue paper covers of the battery jars containing the adults. Strips of that tissue paper with eggs on them were maintained in vials of transparent hard plastic, with a piece of moist tissue paper to maintain approximately 80% relative humidity.

Twenty to 25 g of artificial diet or walnut meats were placed in each 5 × 8.5 cm cylindrical vial of transparent hard plastic. The vials containing the artificial diets were approximately half full, while those containing the walnut meats were less than half full. Depending on the case, 25 or 30 ready - to - hatch eggs or neonate larvae were added to each vial, which was covered with brown tissue paper held tightly with a rubber band, or covered with the opaque white plastic cap of the vial, depending on the treatment. The relative humidity in the rearing rooms and the incubators ranged from ca. 35 to 65%.

The vials containing the larvae were maintained in Percival incubators with temperature and photoperiod control, or in larger constant temperature rooms. In one of those rooms, a number of hardboard dark boxes had their lids open and close at desired hours of the day, through solenoids taking current through time switches. Replicates of each treatment were placed randomly in each such box orncubator and were changed places every few days, when checked for larval development.

The percent water loss in 33 days of artificial diet A in vials covered with brown tissue paper and with their plastic caps, in three rearing rooms was as follows:

Cover of vial	Room 6 21°C	Room 4 26.7°C	Percival incubator No. 2
Brown tissue paper	4.26	5.47	5.42
do.	4.27	5.28	5.76
Plastic cap	0.40	0.92	0.75

When the larvae approached full growth, a strip of corrugated paper was placed so as to line the vial wall, 1 cm above the diet surface, for the larvae to spin their cocoons in. Corrugated paper strips containing fully grown larvae or pupae were removed from the vials every 2 to 4 days and placed in similar clean vials maintained at 26.7°C and L:D 16:8 for the larvae to pupate and adults to emerge. Larvae which did not pupate within 2 weeks under that condition were considered as being in dormancy. The number of fully grown larvae that did not pupate within the first week was also recorded, for additional information on the time needed for pupation in the various treatments.

Wild stock. Fallen walnuts were collected in Riverside, California, on March 20, 1986 under shrubs or under moist dead leaves beneath walnut trees adjacent to a residence. After 2 days in a 10°C refrigerator, the walnuts were cracked and the larvae removed and placed in vials with corrugated paper and a few walnut meats or artificial diet. Most were fully grown, did not feed, and pupated within a week at 26.7°C and L:D 16:8. After pupation, half of them were maintained at L:D 16:8 and half at 14:10 and 26.7°. Emerging adults were maintained under each set of conditions, except for the first 2 days and 3 nights, when they were held at 20°C in a room receiving daylight from a window, to facilitate mating. Eggs were collected and handled as described for the laboratory stock.

Development on various diets

The procedure was as above, with the following additions or modifications: The vials containing the diets received paper strips with 40 ready - to - hatch eggs each and were maintained at 26.7°C and L:D 14:10 or 16:8. Continuous darkness was achieved by wrapping the vials with heavy aluminum foil in the L:D 14:10 room. The corrugated paper strips containing fully grown larvae or pupae were removed from the vials every 2 or 3 days. The number

of pupae in those strips and of emerging adults from the strips or the diets were recorded also every 2 or 3 days, except during two weeks when there were gaps of 6 days between consecutive recordings. Means were compared at the 0.05 level using the t test (Steel and Torrie 1980). Further details are given in the results section.

Results and Discussion

Timeliness of pupation

Laboratory stock larvae on an artificial diet. The generally low incidence of dormancy in this experiment made us decide to present the data not as means and percentages of dormancy, but rather to sum up the numbers of larvae in all replicates and give the actual numbers of larvae in dormancy. In certain vials, mortality during larval growth was unusually high, in contrast to the rest of the same treatment. The data of such vials are presented separately.

It is seen in Table 1 that of the combinations of photoperiod and temperature tested, none caused dormancy in a substantial percentage of fully grown larvae. In only a few vials, where larval mortality was quite high, was there a substantial percentage of larvae in dormancy. Dormancy in those few vials should be attributed to a diseased condition rather than to the respective photoperiod and temperature regimes. In other words, it should be regarded as a delay in pupation or inability to pupate caused by disease rather than as a diapause - mediated dormancy. In conclusion, with the laboratory stock and artificial diet A, no dormancy was caused by long or short photophases in conjunction with relatively high or relatively low temperatures.

TABLE 1. Fully grown larvae of the laboratory stock of *A. transitella* which did not pupate by the end of the 1st and 2nd week at 16L:8D and 26.7°C, when reared under various photoperiod and temperature conditions. Thirty larvae per 25g of artificial diet A per vial.

Photophase (per 24h) and temperature (°C) during		Number of vials	Total number of fully grown larvae	Total number not pupated by end of	
Egg	Larva			1st week	2nd week
0h, 32.2°	16h, 21°	5	111	3	0
	12h, 21°	5	84	3	0
	8h, 21°	5	76	3	0
	0h, 21°	5	79	2	0
	16h, 26.7°	5	91	1	0
	0h, 26.7° for 14 days, then at 12h, 21°	3	61	1	0
	0h, 26.7° for 12 days, then at 12h, 21°	2	43	0	–
	0h, 26.7° for 14 days, then at 8h, 21°	4	78	0	–
0h, 21°	16h, 21°	5	120	1	0
	12h, 21°	6	142	1	0
	8h, 21°	6	119	1	0
	0h, 21°	5	110	0	–
	12h, 21° for 21 days, then at 8h, 21°	2	43	0	–
16h, 26.7°	16h, 26.7°	5	126	0	–
	14h, 26.7°	4	83	0	–
	12h, 21°	2	41	0	–
	8h, 21°	5	111	2	0
	0h, 21°	5	100	0	–
	16h, 21°	1	4	1	1
	12h, 21° for 10 days, then at 0h, 21° for 7 days, then at 12h, 21°	3	58	0	–
	12h, 21° for 14 days, then at 8h, 21°	2	38	1	1
	12h, 21° for 10 days, then at 0h, 21° for 7 days, then at 8h, 21°	3	68	2	0

TABLE 1 continued

16h, 21°	16h, 21°	5	148	7	0
	12h, 21°	3	73	5	0
	do.	1	7	1	1
	12h, 21° for 14 days, then at 8h, 21°	3	57	0	–
	16h, 21° for 7 days, then at 14h, 26.7°	3	65	0	–
	16h, 26.7°	1	33*	5	–
	do.	2	104**	0	–
14h, 26,7°	16h, 26.7°	1	10	0	–
	14h, 26.7°	3	61	0	–
	do.	2	63	2	0
	do.	1	9	6	–
	do.	1	6	4	–
	14h, 26.7° for 10 days, then at 8h, 21°	3	63	0	–
	12h, 21°	3	66	0	–
	14h, 26.7° for 10 days, then at 16h, 26.7°	1	3	4	2
12h, 21°	16h, 26.7°	1	52*	0	–
	14h, 21°	1	21	0	–
	do.	1	8	0	–
	12h, 21°	5	96	2	1
	do.	2	135***	2	–
	8h, 21°	5	116	0	–
	do.	2	133***	3	–
	14h, 26.7° for 10 days, then at 16h, 26.7°	3	42	4	–
	14h, 26.7° for 10 days, then at 14h, 21°	1	4	1	–
	12h, 21° for 14 days, then at 14h, 26.7°	3	47	0	–
12h, 21°	12h, 21° for 14 days, then at 8h, 21°	6	140	2	0
8h, 21°	8h, 21°	5	142	0	–
	do.	2	151***	0	–

* Many eggs per 25 g diet.
** 80-86 eggs per 25 g diet.
*** 120 eggs per 25 g diet.

Laboratory stock larvae on walnut meats. The larvae grew on broken up walnut meats. They were from the same parents and were maintained under the same photoperiod and temperature conditions as those that grew on artificial diet A of the previous section. A considerable proportion of the vials with walnut meats did not yield more than a few fully grown larvae, and the rest yielded considerably fewer grown larvae than the vials with the artificial diet. Furthermore, larval growth was much slower than on artificial diet A. Therefore, dry walnut meats, in addition to their high price, were not a suitable substrate for fast laboratory rearing of the NOW.

Only vials with more than a few fully grown larvae are given in Table 2. It is seen that in five treatments the percentage of larvae that did not pupate by the end of the 2nd week at 26.7° was above 15% and in one of them 40%. In the same five treatments, the percentage of larvae not pupating by the end of the first week was 62-100%. According to our criterion of dormancy, we should conclude that dormancy occurred in some larvae of those five treatments. However, in view of the relatively small number of grown larvae in each treatment, and the fact that no records were kept beyond the 2nd week after full larval growth, we feel that a statement about the occurrence of dormancy in the NOW under the conditions shown in Table 2 should await further work.

On walnut meats, the percentage of larvae that did not pupate by the end of the first week at 26.7° was in general much higher than in larvae fed on artificial diets. It is worth noting

TABLE 2. Fully grown larvae of the laboratory stock of *A. transitella* which did not pupate by the end of the 1st and 2nd week at 16L:8D and 26.7°C, when reared under various photoperiod and temperature conditions. Twenty five to 30 neonate larvae per 30 g of walnut meats per vial.

Photophase (per 24h) and temperature (°C) during		Number of vials	Total number of fully grown larvae	Total number not pupated by end of	
Egg	Larva			1st week	2nd week
0h, 32.2°	16h, 21°	3	14	5	0
	12h, 21°	2	10	9	4
	8h, 21°	3	15	8	1
	0h, 21°	2	24	16	4
	16h, 26.7°	2	21	13	4
	0h, 26.7° for 12 days then at 12h, 21°	1	8	0	0
	0h, 26.7° for 14 days then at 8h, 21°	1	9	9	2
0h, 21°	16h, 21°	2	17	3	0
	12h, 21°	3	7	2	0
	8h, 21°	3	10	4	0
	0h, 21°	3	47	38	8
16h, 26.7°	16h, 26.7°	3	23	10	2
	0h, 21°	3	39	16	0
16h, 21°	16h, 21°	1	3	3	0
	12h, 21°	1	12	0	0
14h, 26.7°	14h, 26.7°	2	24	7	1
8h, 21°	8h, 21°	2	9	2	0

that in 4 out of the 5 treatments that showed dormancy, the eggs were incubated in the dark and at high temperature, and in the 5th treatment again in the dark but at a moderate temperature. High temperature combined with darkness during incubation is known to favor the induction of dormancy in at least two other polyvoltine Lepidoptera (Tzanakakis 1959, Tzanakakis et al. 1988). Therefore, further work to substantiate the occurrence of dormancy in the NOW should include high temperature and darkness during incubation, a variety of larval diets including dried nuts such as walnuts and almonds, and insects recently collected from the wild. Such future work should also include treatments aimed at inducing dormancy in early instar larvae. Such larvae, because of their small size and hiding habits might escape attention.

Wild stock larvae on artificial diets. The number of eggs we obtained from females of the wild stock was relatively small, therefore, the number of treatments was limited and there were no replicates. The treatments were those suggested by the results of the experiment with larvae of the laboratory stock. Two diets were used: the standard one (A), and the fortified one (B). Their composition is given in the Materials and Methods section. In some treatments diet A was packed to ca. 2/3 of its volume (cases marked B in Table 3). In some other treatments the vials were tightly covered with their plastic caps from the day neonate larvae were placed in them (cases marked C and D). The results are given in Table 3.

With all the reservations justified by the lack of replicates in this experiment, we conclude that no dormancy occurred to a substantial percentage of larvae of the wild stock, when reared on the two artificial diets. Same as with larvae of the laboratory stock, a substantial percentage of dormancy in the wild stock occurred only in the few cases where larval mortality was high. We tend to attribute this dormancy again to a diseased condition of the larvae rather than to the photoperiod and temperature conditions involved. The fact that with the wild stock larvae only a long and a relatively long photophase were tried, does not allow any assumptions as to possible conditions that may favor dormancy more than others. On the other hand, the way the laboratory stock insects had been maintained for more than 25 generations,

TABLE 3. Fully grown larvae of a wild stock of *A. transitella* which did not pupate by the end of the 1st and 2nd week at 16L:8D and 26.7°C. Larvae reared on artificial diets A and B. Thirty ready - to - hatch eggs or neonate larvae per treatment. A:larval diet A, unpacked, paper cover on vial. B:diet A, packed, paper cover. C:diet A, unpacked, plastic cover. D:diet B, unpacked, plastic cover.

Photophase (per 24h) and temperature (°C) during		Handling of larval diet	No. pupated within 1st week	Not pupated by the end of	
Parent pupa and egg	Larva			1st week	2nd week
14h, 26.7°	16h, 26.7°	A	16	3	0
		A	16	1	0
		B	12	7	0
		B	22	3	0
		C	7	6	1
		C	19	3	0
		D	17	5	1
		D	15	0	
do.	14h, 26.7°	A	18	0	
do.	8h, 26.7°	B	30	0	
		C	2	4	2
		D	23	0	0
do.	14h, 26.7° for 10 days then at 8h, 21°	B	9	3	2
		C	21	6	1
		D	17	11	1
16h, 26.7°	16h, 26.7°	A	7	1	0
		B	22	0	
		C	24	0	
		D	19	15	0
do.	8h, 26,7°	A	9	1	0
		B	10	4	0
		C	11	0	
		D	11	0	
do.	8h, 21°	B	12	0	
		C	13	3	0
		D	9	4	0
do.	14h, 26.7° for 8 days then at 8h, 21°	B	29	0	
		C	26	0	
		D	25	0	

allowed selection against dormant individuals and slow - growing individuals.

Treating the parents of laboratory stock larvae. The lack of definite dormancy in larvae of the previous experiments, led us to also try certain photoperiod and temperature treatments on late instar larvae of the parental generation in conjunction with certain treatments of their progeny. The insects were of the laboratory stock. Parental larvae were reared on diet A. It is seen in Table 4 that no dormancy occurred under any of the combinations of photoperiod and temperature tried.

Miscellaneous observations. With the laboratory stock and artificial diets, low relative humidity during the incubation of eggs did not result in a picture different from that with 90% or more relative humidity. High humidities were obtained by adding water - soaked tissue paper inside the vials containing the eggs.

In certain treatments not given in the tables, population densities of 5 neonate larvae or eggs per g of artificial diet or of walnut meats did not give more dormant larvae than the 1 larva per g diet density.

Dormant larvae of the laboratory or the wild stock did not spin dense cocoons of the type described by Glick (1922).depending on the cocoon's position in the larval diet, in the corrugated paper or elsewhere in the vial, an adult exit tube was detectable in most cases. No cocoons of unusually dense weave were noticed throughout this work.

TABLE 4. Fully grown larvae of the laboratory stock of *A. transitella* which did not pupate by the end of the 1st and 2nd week at 16L:8D and 26.7°C when they and their parents grew under various photoperiods and temperatures. Three vials of 35 ready - to - hatch eggs per 25g of artificial diet B per treatment.

Photophase (per 24h) and temperature (°C) during		Number of fully grown larvae	Number not pupated by end of	
Parental late larvae*	Filial eggs and larvae		1st week	2nd week
16h, 26.7°	16h, 26.7°	65	7	0
	12h, 26.7°	77	9	1
12h, 21°	16h, 26.7°	81	7	0
	12h, 26.7°	73	8	0
8h, 21°	16h, 26.7°	63	0	
	12h, 26.7°	60	3	0

*Parental pupae at 16h, 26.7°, and adults under natural photoperiod (mid April) and 21°.

Development on soy : yeast diets

In interpreting the results obtained with the soy:yeast diets, we should bear in mind the following: larvae that pupated within the diet were not recorded as fully grown larvae nor as pupae. They were recorded only as adults. This is why the number of adults in certain treatments, as shown in the table, exceeds the number of fully grown larvae. Therefore, no conclusions should be drawn as to larval and pupal survival by comparing the numbers of individuals recorded in the previous developmental stage. The same holds for the number of days needed for 50% of larvae to complete growth or to pupate. Rough comparisons can be made taking into consideration that the mean time from full larval growth to pupation was 3 to 4 days and from full larval growth to adult emergence 13 days. The number of eggs that hatched was not recorded, but in a few vials of another experiment the percent egg hatch was from 60% to over 85%. Therefore, yields in grown larvae or adults shown in the table may differ substantially from the true ones.

Soy : yeast ratios. Not a single larva completed development on straight brewers' yeast powder under any of the conditions shown in Table 5. Therefore, straight yeast is not included in the table. Straight soybean flour, or 9:1 and 7:3 soy:yeast mixtures allowed the production of fully grown larvae, pupae and adults of normal appearance. As seen in Table 5, at 26.7°C and L:D 14:10 in vials with tissue paper covers (Treatments A to D), the yield in grown larvae and adults was poor in the straight soy, better in the 9:1 soy:yeast diet and even better in the 7:3 diet and the reference diet B, although the differences were not statistically significant between most of those treatments. It is worth noting that the yield of the 7:3 diet was not significantly different from that of the reference diet. When the vials had plastic covers (Treatments E to I) the yields between the four diets did not differ significantly.

As to the rate of larval growth, we see that the addition of yeast to the soy reduced the rate significantly and that the reference diet gave by far the fastest growth. The picture is generally similar with plastic covers of the vials, although some differences were not significant. Although a slower growth indicates a poorer diet, such growth may have some use in certain experiments such as those aiming at detecting the possible occurrence of dormancy. It may also be desirable for periods when we need only to maintain a given colony with the minimum of labor and cost.

With all the reservations imposed by the method we used, we could conclude that straight soybean flour or mixtures of it with 10% or 30% brewers' yeast powder allow the production of adults of the navel orangeworm. In view of the fact that soy:yeast diets are simple ones, their further testing is justified to establish whether they can be relied upon for continuous rearing of this insect. Forms other than the powder one, such as pellets or flakes should also be tested.

Effect of limited aeration. Here we assume that aeration inside vials with tight - fitting lids of plastic was limited, as compared to vials which had tissue paper covers. The tight - fitting lid remained in place till we observed the first larvae approaching full growth. The vials were then opened for a short time to

TABLE 5. Yield in and rate of growth of navel orangeworms at 26.7°C on soy flour and brewers' yeast diets, under various photoperiod and humidity conditions (40 eggs per vial).

Test	Number of vials	Soy:yeast ratio	Mean percent yield ± S.D. in		Mean no. of days ± S.D. to 50% of	
			Grown larvae*	Adults	Grown larvae*	Adults
			Paper cover of vials, L:D 14:10			
A	4	10:0	10.5± 5.2 a	4.0±4.3 a	40.7±0.5 a	48.0±3.0 a
B	5	9:1	16.2± 8.3 ab	8.0±4.7 bc	44.8±2.5 be	57.0±2.3 b
C	5	7:3	21.0± 5.0 bc	8.6±3.0 bc	51.6±2.5 c	65.4±5.1 c
D	2	Diet B	21.5± 2.1 bd	12.0±5.7 cd	25.0±0.1 h	29.0±4.2 d
			Plastic cover of vials, L:D 14:10			
E	3	10:0	25.0± 2.6 cd	12.3±4.6 cd	45.7±4.0 bg	61.0±7.2 bc
G	3	9:1	28.0±16.4 cd	11.3±5.0 cd	48.3±3.5 dfg	57.7±5.1 b
H	5	7:3	26.6±12.4 cd	12.2±5.4 d	52.2±6.1 cd	65.6±8.5 c
I	3	Diet B	21.7±13.4 acd	7.3±8.4 abd	22.0±5.2 hi	30.7±3.8 d
			Plastic cover of vials, L:D 16:8			
K	3	10:0	30.7±19.8 bcd	9.0±4.0 abd	43.7±5.1 aefg	61.0±7.2 bc
L	3	9:1	31.0± 8.5 d	13.0±4.4 d	49.3±5.1 cdg	69.0±0.1 c
M	3	7:3	23.7± 5.1 cd	9.3±3.1 bd	50.0±5.6 cdg	69.0±4.0 c
			Plastic cover of vials, L:D 0:24			
N	3	10:0	13.3± 7.4 ae	1.3±1.2 a	65.7±5.8 k	76.0±9.9 ce
P	3	9:1	15.7± 8.5 abc	0.7±1.2 a	76.3±5.8 m	83.0±0.1 e
Q	3	7:3	11.0± 4.0 ae	1.0±1.0 a	70.3±2.3 km	76.0±9.9 ce
			Plastic cover of vials, high RH, L:D 14:10			
R	4	10:1	0	0	–	–
S	1	do.	10.0	4.0	25.0	38.0
T	5	9:1	14.4± 8.8 ab	8.2±2.4 bc	25.8±1.8 h	39.0±0.1 f
U	1	do.	29.0	10.0	21.0	38.0
V	5	7:3	14.8± 4.6 be	13.0±5.1 d	25.0±2.8 h	39.3±3.0 f
W	2	Diet B	16.0± 5.7 abd	14.0±2.8 d	16.0±0 i	29.0±0.1 d

* Means within the same column followed by the same letter are not significantly different at the 0.05 level.

add a strip of corrugated paper to each for the larvae to pupate in. From then on, the vials were opened every 2 or 3 days to renew the corrugated paper and/or to remove grown larvae or adults. Thus, in every such vial the condition which we consider as of limited aeration prevailed till the first fully grown larvae were observed. As seen in Table 5 (Treatments E to I), in the soy and the soy:yeast diets limited aeration resulted in a better yield in grown larvae and adults as compared to vials with more aeration (A to D). In general, limited aeration did not affect significantly the rate of larval growth either.

Effect of illumination. With a tight-fitting lid, the yield in grown larvae and adults under a L:D 16:8 photoperiod (Table 5, K to M) was generally not significantly different from that under a L:D 14:10 (E to I). By contrast, under continuous darkness (N to Q) the yield as well as the rate of larval growth were significantly lower than in the presence of light, being the lowest recorded in this experiment.

Effect of high humidity. High humidity in the vials during larval growth was achieved by fastening a 1 cm piece of water-soaked cotton wick on the inside of the vial's lid. The wick remained moist throughout the time necessary for the larvae to reach full growth. Relative humidity on the surface of the diets must have been at or near 100%. The high humidity favored the excessive growth of fungi on and in the soy and soy:yeast diets but not on the reference diet B. Thus, the yield and growth rate data from the high humidity vials include the possibility that larvae fed also on fungal hyphae and spores. Therefore, data from high humidity vials should not be taken as showing the effect of humidity only.

As seen in Table 5 (R), the yield on straight soy was nil. Yet, an extra vial (S) in which folded tissue paper in contact with the surface

of the diet was added from the day the eggs were added, did not develop obvious fungal growth and gave a few grown larvae, pupae and adults. This favorable effect of folded tissue paper was also noticed in the extra vial (U) with the 9:1 soy:yeast diet. The yield in grown larvae of the soy:yeast diets under high humidity (T,V) was significantly lower than under no such condition (G, H). This was not the case with the reference diet. On the other hand, the yield in adults was not significantly affected by high humidity. Under high humidity the rate of larval growth on the two soy:yeast diets (T, V) was significantly and substantially faster than without high humidity (G, H). In fact, the larval stage under high humidity was completed in approximately half the time it took under no such condition. Thus, the number of days to full larval growth compares favorably with that obtained under the same temperature on a satisfactory artificial larval diet such as the one of Finney and Brinkman (1967), or on dehisced almonds (Sanderson 1986). The rate of larval growth on diet B was under high humidity the fastest recorded in the present experiment, although it did not differ significantly from that under no high humidity.

The navel orangeworm is known to do best under rather moist conditions (Michelbacher and Davis 1961, Sparks 1964). In nature, larvae often develop inside fruits infected by fungi. It would be interesting to test the growth of this insect under a range of relative humidities in conjunction with the presence of certain fungi in the larval diet. The fungi should preferably be among those associated with fruits the insect infests in nature.

Development on walnuts and walnut meats

In a preliminary experiment without replicates, the suitability of broken up walnut meats was compared with whole nuts for larval development. The walnuts were from the local market. Their shells were carefully cracked, then the nuts placed in vials in such a way that the shells remained in place without leaving the meats exposed. Certain nuts were used dry while others, after cracking the shells, were immersed in tap water for 3 hours for their inside to be soaked, then they were strained for 1 hour on tissue paper and placed in the vials. Meats from the same batch of walnuts were broken up by hand so that 10 to 15 main pieces were derived from each nut. Two walnuts or 25 g of meats were placed in each vial, together with 16 neonate larvae of the NOW. In half the vials brewers' yeast powder was dusted on the bottom of the vial and on the surface of the nuts and the meats to possibly help neonate larvae, by offering them an additional starter food. This was carried out before we knew that straight yeast powder was not a suitable food for larvae of this species.

The neonate larvae emerged from eggs laid by adults of the laboratory colony in late March 1986 at 21° and natural photoperiod. They were incubated at 26.7° and L:D 14:10. The larvae developed at 26.7° and L:D 16:8 for the first 18 days, then under the same temperature and a L:D 8:16 photoperiod for another 35 days. The vials were checked weekly. When the most developed larvae approached full growth, a strip of corrugated paper was added to each vial to provide extra pupation sites. On the 53rd day the entire nuts and the meats were searched and all stages of the insect recorded.

It is seen in Table 6 that survival was maximal in the dry nuts, less in the broken up meats and least in the water soaked nuts. The number of fully grown larvae plus pupae was also the least in the soaked nuts. The addition of brewers' yeast powder did not improve either the survival or the rate of larval development. Water soaking of the nuts did not improve the rate of larval development either. Using a different method (filling nut shells with crushed walnut meats and whole meats), a much higher larval population density and continuous illumination, Sparks (1964) had better larval and pupal survival on entire walnut meats than on crushed ones.

An interesting observation of this small experiment is that while 50-81% of the individuals in the entire nuts had completed larval growth and some had pupated, a small percentage of larvae was still in the 4 - 6 mm size. Sparks (1964) also had a wide variation in the rate of development of NOW growing on entire walnut meats inside the shells. Therefore, the reasons of delayed development of certain larvae on whole meats of walnuts and possibly also of other nuts should be looked into. Is it that inside the entire nuts certain larvae are forced to feed less than the rest, or do some smaller larvae exhibit some kind of dormancy?

TABLE 6. Number of immature stages of *A. transitella* of the laboratory stock, recorded on the 53rd day of growth at 26.7° and 16L:8D. Two entire walnuts or 25g of meats with 16 neonate larvae in a vial per treatment.

Diet	Pupae	Larvae			Total larvae and pupae	Percent fully grown larvae plus pupae
		Fully grown	7-10mm long	4-6mm long		
Walnut meats*	2	5	4	0	11	64
Dry cracked walnuts	5	8	0	3	16	81
Water-soaked cracked walnuts	5	0	1	1	7	71
Walnut meats, dusted with yeast	4	3	3	0	10	70
Dry cracked walnuts, dusted with yeast	3	3	3	3	12	50
Water-soaked cracked walnuts dusted with yeast	5	0	2	1	8	62

* Live adults of *Tribolium* sp. were also found among the meats on day 53 in this treatment.

What happens inside intact uncracked walnuts or almonds on the trees or fallen to the ground? Further work to reveal the larval age structure inside nuts throughout the year and the factors that determine it is certainly justified.

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KEY WORDS: *Amyelois transitella,* Navel orangeworm, Diapause, Insect dormancy, Rate of insect development, Illumination effects, Humidity effects

Προνυμφική Ανάπτυξη και Εγκαιρότητα Νύμφωσης στο Εργαστήριο του *Amyelois transitella* (Walker) (Lepidoptera: Phycitidae), σε Ορισμένες Τροφές και σε Διάφορες Συνθήκες Φωτοπεριόδου, Θερμοκρασίας, Αερισμού και Υγρασίας

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ΠΕΡΙΛΗΨΗ

Το *Amyelois transitella*, ενώ είναι κυρίως σαπροφάγο, αποτελεί και σοβαρό εχθρό των αμυγδάλων, καρυδιών και φιστικιών στην Καλιφόρνια και ορισμένες άλλες περιοχές της γης. Διαχειμάζει ως προνύμφη διαφόρων ηλικιών στους μουμιοποιημένους καρπούς που μένουν στα δέντρα ή που έχουν πέσει στο έδαφος. Την άνοιξη, τα ενήλικα ωοτοκούν σε μουμιοποιημένους καρπούς και αργότερα σε καρπούς της νέας εσοδείας όταν το μεσοκάρπιό τους σχίζεται και αρχίζει να αποκολλάται από το ενδοκάρπιο. Ορισμένοι ερευνητές συμπεραίνουν ότι το έντομο δεν παρουσιάζει διάπαυση στην Καλιφόρνια, ενώ άλλοι το αντίθετο.

Με σκοπό να προκαλέσουμε διάπαυση, μελετήσαμε την ανάπτυξη προνυμφών του εντόμου σε διάφορες φωτοπεριόδους, θερμοκρασίες και τροφές. Αυγά που ήταν έτοιμα να εκκολαφθούν ή νεαρές προνύμφες, τοποθετήθηκαν σε διαφανή κυλινδρικά δοχεία που είχαν ως τη μέση τους τροφή. Μετά την πλήρη ανάπτυξή τους, όσες προνύμφες δεν νυμφώθηκαν μέσα σε 14 ημέρες σε 26,7°C και φωτοπερίοδο (L:D) 16:8 ωρών θεωρήθηκε ότι ήταν σε διάπαυση.

Όταν οι προνύμφες έφαγαν τεχνητές τροφές που περιείχαν πίτουρα σιταριού, ζυθοζύμη και βιταμίνες, και που ενισχύθηκαν ή όχι με πλούσιο σε πρωτεΐνη σπόρο δημητριακών και κρόκο αυγού, καμιά από τις συνθήκες που δοκιμάστηκαν δεν προκάλεσε διάπαυση σε αξιόλογο ποσοστό προνυμφών είτε της εργαστηριακής αποικίας είτε του άγριου πληθυσμού που είχαμε πρόσφατα συλλέξει από το ύπαιθρο. Όταν οι προνύμφες αναπτύχθηκαν τρώγοντας ξερή ψύχα καρυδιών, η ανάπτυξή τους ήταν βραδύτερη και η επιβίωση μικρότερη από ότι με τεχνητές τροφές. Με καρυδόψυχα, όταν τα αυγά του εντόμου επωάστηκαν σε 32,2° και L:D 0:24, τα ακόλουθα ποσοστά αναπτυγμένων προνυμφών της εργαστηριακής αποικίας εκδήλωσαν διάπαυση, όταν οι προνύμφες αναπτύχθηκαν στις εξής συνθήκες: 40% σε 21° και L:D 12:12, 17% σε 21° και L:D 0:24, 19% σε 26,7° και L:D 16:8. Επίσης, 22% των προνυμφών διέπαυσε όταν αναπτύχθηκαν σε 26,7° και L:D 0:24 τις πρώτες 14 ημέρες και στη συνέχεια 21° και L:D 8:16 την υπόλοιπη προνυμφική ζωή. Όταν και τα έμβρυα και οι προνύμφες αναπτύχθηκαν σε 21° και L:D 0:24, 17% των προνυμφών εκδήλωσαν διάπαυση. Εν τούτοις, ο σχετικά μικρός αριθμός αναπτυγμένων προνυμφών στις ομάδες που έφαγαν καρυδόψυχα, δείχνει ότι χρειάζεται περαιτέρω έρευνα πριν αποδειχτεί η ύπαρξη διάπαυσης στο έντομο αυτό.

Καμμιά προνύμφη δεν αναπτύχθηκε σε σκέτη κονιοποιημένη ζυθοζύμη. Σκέτο σογιάλευρο, ή μίγματά του με ζυθοζύμη σε αναλογίες 9:1 και 7:3 επέτρεψαν την παραγωγή

αναπτυγμένων προνυμφών, νυμφών και ενηλίκων που φαίνονταν κανονικά. Η ταχύτητα ανάπτυξης των προνυμφών στα μίγματα σογιαλεύρου: ζυθοζύμης ήταν σημαντικά μικρότερη από ότι στην τεχνητή τροφή αναφοράς.

Για εκτροφή σε 26,7°, φωτοπερίοδος L:D 16:8 ήταν εξ ίσου καλή με 14:10. Συνεχές σκοτάδι έδωσε σημαντικά μικρότερη απόδοση σε ενήλικα και βραδύτερη προνυμφική ανάπτυξη.

Υψηλή σχετική υγρασία στην επιφάνεια της τροφής, επέτρεψε υπερβολική ανάπτυξη μυκήτων επάνω και μέσα στα μίγματα σογιαλεύρου: ζυθοζύμης και έδωσε πολύ ταχύτερη ανάπτυξη των προνυμφών.

Οι προνύμφες αναπτύχθηκαν καλά σε ξερή καρυδόψυχα και μέσα σε σπασμένα ξερά ή μουσκεμένα καρύδια. Μέσα στα καρύδια, η ταχύτητα ανάπτυξης των προνυμφών ήταν ανομοιόμορφη. Ορισμένες προνύμφες ήταν ακόμα σχετικά μικρές την 53η ημέρα ανάπτυξης σε 26,7°, ενώ οι πλείστες είχαν συμπληρώσει την ανάπτυξή τους ή είχαν ήδη νυμφωθεί.