Comparative Analysis of Two Rearing Procedures for Diamondback Moth (Lepidoptera: Plutellidae)[1]

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ABSTRACT Rape seedlings, *Brassica napus* L., and a wheat germ-based artificial diet were compared as media for rearing diamondback moth (DBM), *Plutella xylostella* (L.), for six generations. Mean pupal weight and total number of eggs laid per female were always greater when larvae were reared on artificial diet; however, percentage of eggs hatching was usually greater and development time usually shorter when larvae were reared on rape seedlings. High larval survivorship (>70%) could be obtained on either media. Larvae which were reared on artificial diet were consistently more susceptible to the insecticides methomyl and permethrin, indicating potential problems in using artificial diet for insecticide studies. When larvae were reared on either medium for six generations and then transferred to cabbage, larval survivorship was nearly equal, indicating that either method could be used for artificially incoculating plants for host plant resistance studies. Although it was easier and cheaper to rear DBM on artificial diet, recommendations for using one rearing method over the other must be based on the ultimate use of the colony.

KEY WORDS *Plutella xylostella,* diamondback moth, rearing, artificial diet, rape seedlings.

The diamondback moth (DBM), *Plutella xylostella* (L.), has become the major pest of crucifers throughout the world and has become especially severe in tropical areas where it has often developed resistance to most available insecticides (Talekar and Griggs, 1986). Georghiou (1981) reports DBM resistance to 36 insecticides in 14 countries. In a recent survey of insecticide resistance in North America, Shelton and Wyman (unpublished) found high levels of resistance in DBM to Ambush (>80 fold) and methomyl (>780 fold).

Plant resistance, biological control, and sterile insect technique are three alternatives to insecticidal control of DBM currently being developed. All three of these approaches require mass rearing of DBM. Hou (1986) reviewed the literature on mass rearing of DBM and noted that two of the most promising rearing methods were a wheat germ-based artificial diet, originally adapted for rearing DBM by Biever and Boldt (1971), and a rape seedling diet, *Brassica napus* L. (Koshihara and Yamada 1976). Although Hou (1986) concluded that a wheat germ-based diet would be preferable for most applications, no systematic study compared these two diets for DBM. Existing reports compare colonies from different locations, but these comparisons may be invalid because of variations due to

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subtle differences in diet preparation, variations in the insects, or other factors unqiue to each location (Hsiao and Hou 1978).

The usual criteria for evaluating diets are development time, pupal weight, survivorship, oviposition rates, and rearing costs. However, the choice of rearing method also depends on the intended experimental use of the colony. In the case of insecticide resistance studies, it often takes at least two generations of laboratory rearing to build the wild colony to a size suitable for insecticide assays. It is crucial that a laboratory rearing method be selected that enhances the rapid buildup of the colony but still maintains natural insecticide susceptibility. For host plant resistance studies it is essential that insects will readily transfer from the rearing diet to test plants and feed and develop normally.

To provide a more objective comparison of the wheat germ-based and rape seedling diets for rearing DBM we compared the two methods on a single population. Specifically, we compared: 1) developmental and reproductive rates of DBM reared on artificial diet and rape seedlings; 2) influences of each rearing method on the susceptibility of DBM to two commonly used insecticides, and; 3) the ability of DBM from each rearing method to transfer to and develop on cabbage plants.

Materials and Methods

Prior to the first killing frost, ca. 500 DBM larvae and puape were collected on 1 November, 1988 from the New York State Experiment Station Robbins Farm, Geneva, NY. Insects were collected from insecticide-free cabbage, cv 'Roundup,' and returned to the laboratory for rearing. Pupae were refrigerated at 4° C while larvae were allowed to feed on cabbage leaves in a rearing room with conditions set at 24 ± 2° C, 16:8 L:D, and RH 50 ± 5% until pupation. When all insects had pupated, pupae were combined and placed in oviposition cages (approximately 50 pupae per cage) made of clear plastic tubes (15 cm long × 12 cm dia.). The ends of the tubes were capped with a plastic plug, with three quarters of the plug cut out and the opening covered with an organdy cloth. Oviposition cages were placed in a rearing room with conditions the same as above. Adults were allowed to emerge, mate, and oviposit. Adults were fed a 10% sucrose solution distributed on cotton dental wicks. A single aluminum foil strip, 2.5 cm × 7.5 cm, was provided as an oviposition substrate. The foil strip was crumpled to create suitable ovipositional ridges and depressions, then smoothed to a flat surface, and finally dipped into autoclaved cabbage juice (65 g cabbage in 500 ml water) to increase oviposition. Eggs were collected from the oviposition cages and used to start two new colonies, one raised on artificial diet and the other on rape seedlings. The two rearing methods were set up simultaneously and all rearing was done in the same chamber with the environmental conditions the same as above.

Rearing Methods

Rape Seedlings. Rape seedlings, *Brassica napus,* cv Dwarf Essex, were grown in plastic containers (30 cm long, 13.3 cm wide and 6.5 cm deep). Paper toweling was used to cover the top. Wetted Cornell Mix [vermiculite, peat moss, green limestone, and fertilizer (unimix 10-20-5)] was placed in the bottom of the container to a depth of 3.5 cm. One teaspoon of rape seedlings was spread on top of the Cornell mix and dry Cornell mix was sprinkled over the seeds to absorb the excess

moisture from the bottom layer. The containers were placed in a greenhouse (20 ± 5° C and with artificial light at 16:8 L:D) for ca. 4 d, by which time the rape seedlings had grown to a height of 6 cm. Each of the twenty containers of rape seedlings (ca. 300 seedlings/100 cm^2) were inoculated with 75 eggs from the F_1 generation. Using these same techniques, but with 6-10 containers per generation for generations 2 - 6 developmental parameters (as described below) were documented.

Artifical diet. We used a wheat germ-based artificial diet modified from the original diet by Biever and Boldt (1971) (see Appendix 1 for diet ingredients and standard procedures for preparation). Artificial diet was prepared the day before inoculation and allowed to cool overnight. Sixteen ounce (ca. 470 ml) styrofoam cups (Dart 16MJ32, Dart Container Corporation, Mason, MI) were filled with approximately 87 ml of artificial diet. Twenty of these cups were subsequently inoculated with 75 DBM eggs each from the F_1 generation, then covered with opaque lids (Dart 32JL). Using these same techniques, but with 6 - 10 cups per generation for generations 2 - 6, developmental parameter (as described below) were documented.

Developmental Parameters

Egg hatch rate, development time, pupal weight, sex ratio and larval survivorship. The percentage of eggs hatching for each rearing procedure was determined by removing the egg sheet (which initially had 75 eggs) from the rearing container (either diet cups or containers) when the larvae reached second instar. Since an empty egg case was left behind for each larva that had successfully emerged, the total number of eggs that hatched was easily determined. The mean hatch rates and standard errors were calculated and analyzed using a two-sample *t*-test (SAS Institute 1985).

All the eggs used in a rearing unit were < 24 hrs old. The time from egg to pupa was monitored by examination for the first pupa in each unit. Once pupation began, the number of puape per day per rearing unit was recorded and each pupa was weighed. The means and standard errors for development time and pupal weight for each rearing method were calculated and compared for every generation using a nested design GLM procedure (SAS Institute 1985). Since data were collected from several rearing cups for each rearing method, a nested design analysis was used to eliminate any statistically significant effects due to the cups. When pupae were collected, they were sexed to determine the sex ratio for that generation.

Larval survivorship was calculated by dividing the total number of pupae by the total number of eggs hatched. Egg to pupal survivorship was calculated as % larval survivorship X egg viability. The means and standard errors were calculated and analyzed using a two-sample *t*-test (SAS Institute 1985).

After all the above data were recorded for the first generation, the pupae from all rearing units of a single rearing method were combined and set up for another generation, with environmental conditions the same as above. This was repeated for the next five generations.

Oviposition rates. For each generation, ten males and ten females from each rearing method were placed into each of seven oviposition cages (as above) per rearing method. Adults emerged, mated, and laid eggs on aluminum foil oviposition strips. Oviposition strips were collected daily, and the number of eggs were counted and recorded. The total number of eggs laid per cage was then divided by

the total number of females per cage (10). These data were used to determine the number of eggs laid per female in each generation and also the time of peak oviposition. At the time of peak oviposition, the eggs were collected from all ovipositional cages of a rearing method and combined. These combined eggs were then used to set up the next generation as stated previously.

Insecticide Susceptibility

Leaf dip insecticide assays were used to detect differences in insecticide susceptibility on each rearing method over several generations. The two insecticides used were permethrin (Ambush® 2E) and methomyl (Lannate® 1.8L) tested at the following concentrations (mg AI/ml); permethrin: 5.62, 1.78, 0.56, 0.18, 0.056, and 0.018; methomyl: 100.0, 17.78, 3.16, 0.56, 0.10, 0.018 (field rates for DBM for permethrin and methomyl are 2.5 and 8.2 mg AI/ml, respectively). Five drops of BOND® sticker spreader (Loveland Industries, Loveland, CO) per 1000 ml H_2O were added to each of the above concentrations. There were three replicates of each concentration and an untreated control (distilled water and sticker spreader). Cabbage leaves from the outer layers of the head were cut into 6 cm dia. disks which were dipped into an insecticide mixture for 5 seconds, held vertically to allow excess solution to drip off, and placed on a rack to dry. After two hours drying time, the disks were placed in a petri dish with a pre-moistened filter paper on the bottom to prevent desiccation of the cabbage leaf disk. Ten third instar larvae (ca. 1 mg) were placed in each petri dish. Larval mortality was assessed after 48 hrs (larvae were considered dead if they did not move when prodded). Data from these assays were analyzed using POLO Probit analysis (Russell et al. 1977). Insecticide assays were performed at the 3rd, 4th, and 6th generations for permethrin, and at the 1st, 3rd, 4th, and 6th generations for methomyl. It was not feasible to run assays for every generation as the assays greatly reduced the population each time.

Transfer to Cabbage

After the sixth generation, pupae were collected from each rearing method and set up in ovipositional cages as before. For both rearing methods, six foil strips of 100 eggs were placed on each of six cabbage plants, cv 'Roundup.' Cabbage plants were in the heading stage and grown in pots in the greenhouse. Eggs were allowed to hatch and larvae to feed on the plants until pupation. Time to first pupa was recorded, after which pupae were removed on a daily basis and weighed. Values for egg to pupa survivorship, development time from egg to pupa, and pupal weight for each rearing method were calculated and compared using a nested design GLM (SAS Institute 1985).

Results

Developmental parameters

Egg hatch rate, development time, pupal weight, sex ratio, and larval survivorship. In four of six generations there were no significant differences in percentage of eggs hatching between the two rearing methods (Table 1). In the two generations with significant differences, artificial diet had a lower percentage of eggs hatching. Overall, the percentage of eggs hatching for artificial diet was 61.4, and 74.5 for rape seedlings.

Table 1. Summary statistics for relative fitness of DBM reared on artificial diet vs. rape seedlings.

	Mean Values ± S. E.					
	Pupal Weight (mg)	Hatch Rate (%)	Larval Survivorship (%)	Egg to Pupa* Survivorship (%)	Development Time (days) Egg to Pupae	Eggs/ Female/ Generation
1st Generation						
Artificial diet	8.88 ± 1.06a †	77.0 ± 5.3a	34.1 ± 5.0a	26.0 ± 4.0a	21.5 + 0.12a	11.7 ± 2.6a
Rape seedlings	5.59 ± 0.38b	73.4 ± 4.6a	33.7 ± 5.3a	24.0 ± 3.7a	17.2 + 0.05b	13.4 ± 4.2a
2nd Generation						
Artificial diet	9.10 ± 1.00a	21.7 ± 5.8a	14.4 ± 5.1a ‡	4.4 ± 1.4a	18.1 + 0.70a	160.5 §
Rape seedlings	7.37 ± 0.26b	61.4 ± 4.4b	79.4 ± 3.6b	54.4 ± 4.0b	15.9 + 0.06b	135.0
3rd Generation						
Artificial diet	7.90 ± 0.20a	69.3 ± 8.7a	79.5 ± 6.7a	54.2 ± 6.8a	15.6 + 0.04a	97.1 ± 12.5a
Rape seedlings	5.87 ± 0.09b	70.3 ± 3.7a	71.4 ± 4.5a	50.6 ± 2.7a	14.7 + 0.06b	43.5 ± 12.2b
4th Generation						
Artificial diet	6.66 ± 0.14a	73.0 ± 3.8a	64.7 ± 6.9a	46.0 ± 4.3a	15.7 + 0.07a	113.0 ± 8.5a
Rape seedlings	4.96 ± 0.10b	78.6 ± 5.5a	64.1 ± 7.5a	51.2 ± 6.9a	15.9 + 0.08a	49.9 ± 11.6b
5th Generation						
Artificial diet	6.48 ± 0.15a	69.9 ± 5.4a	71.1 ± 2.6a	50.3 ± 4.9a	14.8 + 0.09a	79.8 ± 16.7a
Rape seedlings	4.59 ± 0.21b	85.3 ± 5.4a	34.9 ± 8.0b	30.3 ± 8.3a	14.0 + 0.10b	45.7 ± 7.1a
6th Generation						
Artificial diet	6.65 ± 0.32a	57.4 ± 6.5a	32.3 ± 4.3a	18.5 ± 3.1a	17.1 + 0.03a	16.8 ± 5.2a
Rape seedlings	5.09 ± 0.08b	77.9 ± 4.5b	74.1 ± 5.8b	56.5 ± 2.8b	16.1 + 0.02b	47.3 ± 10.6b

* Percent survivorship is calculated by dividing the total number of pupae by the total number of hatched eggs. Egg to pupal survivorship is calculated using % larval survivorship X hatch viability.

† Values followed by same letter within a generation are not significantly different at p = 0.05.

‡ Low survivorship due to mold contamination.

§ Insufficient data to perform statistical analysis.

In all but the fourth generation, development times (egg to pupa) were significantly less ($p \leq 0.05$) for insects reared on rape seedlings. In the fourth generation, development times were nearly identical. Development times ranged from 14.0 to 17.2 days for insects reared on rape seedlings, and 14.8 to 21.5 days for insect reared artificial diet.

Pupal weight was significantly greater $p \leq 0.05$) in all generations of DBM larvae reared on artificial diet. Pupal weights ranged from 4.59 to 7.37 mg for larvae reared on rape seedlings, and 6.48 to 9.10 mg for larvae reared on wheat germ-based artificial diet (Table 1).

In generations three through five, there were always more males than females when reared on artificial diet, and usually more females than males when reared on rape seedlings. Percentages of males range from 51 to 59 percent on artificial diet, and from 40 to 55 percent on rape seedlings. Sex ratio was not determined for generations one, two, and six.

Larval survivorship was low (33.7 - 34.1%) on both rape seedlings and artificial diet in the first generation. Survivorship on artificial diet in the second generation was even lower (14.4%), but some of this was attributed to fungal contamination. In subsequent generations, survivorship increased reaching a level of 79.5% in generation three for insects reared on artificial diet, and 79.4% in generation two for insects reared on rape seedlings (Table 1). Egg to pupal surviorship reached a level of 54.2% in generation three for insects reared on artificial diet, and 56.5% in generation six for insects reared on rape seedlings (Table 1).

Oviposition rates. The oviposition rate for females in the first generation was low for both rape seedlings and artificial diet: 11.7 and 13.4 eggs per female, respectively. In the second generation, the oviposition rate increased over 10-fold; 160.4 and 135.0 eggs per female for rape seedlings and artificial diet, respectively (Table 1). With the exception of generations one and six, the number of eggs per female per generation was higher for insects reared on artificial diet. Peak oviposition on artificial diet always occurred on the first day of the oviposition period, except in generation six in which the peak occurred on the second day. On rape seedlings, peak oviposition occurred within the first two days of the oviposition period, except generation one (peak at 4 days) and generation five (peak at 5 days).

Insecticide Susceptibility

DBM larvae reared on artificial diet were more susceptible to permethrin (2.4 to 37.0 fold) and methomyl (3.1 to 20.7 fold) than larvae reared on rape seedlings (Table 2). Additionally, there was a trend for later generations in both rearing method regimes to be more susceptible than earlier generations. For diet-reared and rape seedling-reared DBM tested against methomyl, there was a 72.7 and 11.0 fold reduction respectively, in susceptibility from generations 1 to 6, and a 2.7 and 15.0 fold reduction, respectively, against permethrin from generations 3 to 6.

Transfer to Cabbage

Egg to pupal survivorship was not significantly different for insects reared on rape seedlings (49.6%) or artificial diet (51.1%) when they were transferred to cabbage after 6 generations (Table 3). Although the difference was not significant, pupal weights were greater for insects that were reared on artificial diet and then transferred to cabbage, 5.15 mg, than for insects reared on rape seedlings and then

Table 2. Susceptibility of diamondback moth larvae to permethrin and methomyl when larvae are reared on either artificial diet or rape seedlings.

		PERMETHRIN		
	Generation	Slope ± SE	LC_{50}*	90% FL(LC_{50})†
Artificial diet	3	1.471 ± 0.252	0.032	0.020 - 0.047
Rape seedlings	3	1.177 ± 0.178	1.185	0.594 - 2.547
Artificial diet	4	2.290 ± 0.294	0.207	0.094 - 0.483
Rape seedlings	4	1.582 ± 0.185	0.488	0.208 - 1.248
Artificial diet	6	2.348 ± 1.040	0.012	NA‡
Rape seedlings	6	0.457 ± 0.118	0.080	NA
		METHOMYL		
	Generation	Slope ± SE	LC_{50}*	90% FL(LC_{50})†
Artificial diet	1	0.895 ± 0.135	8.950	5.192 - 19.701
Rape seedlings	1	0.451 ± 0.092	28.129	5.457 - 2567.0
Artificial diet	3	1.267 ± 0.161	0.401	0.230 - 0.698
Rape seedlings	3	0.823 ± 0.120	5.083	1.561 - 49.063
Artificial diet	4	1.510 ± 0.199	0.548	0.378 - 0.797
Rape seedlings	4	1.695 ± 0.247	9.121	6.438 - 13.080
Artificial diet	6	1.144 ± 0.232	0.123	0.060 - 0.230
Rape seedlings	6	1.531 ± 0.204	2.551	1.769 - 3.706

* LC_{50} values (lethal dose required to kill 50% of the population) in mg AI/ml and calculated using POLO Probit analysis (Russell et al. 1977).

† Fiducial Limits at 90%.

‡ Value not generated by POLO Probit analysis.

Table 3. Summary statistics for the relative fitness of DBM reared on artificial diet vs. rape seedlings after eggs are placed back onto cabbage leaves.

	Egg to Pupa Survivorship (% ± S.E.)	Pupal Weight (mg) (± S.E.)	Development Time Egg to Pupa (Days ± S.E.)
Artificial diet	51.1 ± 6.2a*	5.15 ± 0.91a	17.8 ± 0.10a
Rape seedlings	49.6 ± 4.4a	3.58 ± 0.50a	16.5 ± 0.09b

* Values followed by same letter within a column are not significantly different at p = 0.05.

transferred to cabbage, 3.58 mg. Both these values were lower than any of the six previous generations when insects were reared on rape seedlings or artificial diet. Development time on cabbage was significantly less for insects reared on rape seedlings than insects reared on artificial diet (difference of >1 day), and this was consistent with the results from generations one through six. Development time on cabbage for insects from each rearing method was very similar to that of the previous six generations.

Discussion

Each method for rearing DBM has its advantages and disadvantages, thus choice of method should be based on the intended use of the colony. Regardless of which rearing method is chosen, high larval survivorship (>70%) can be obtained. Considerable variation in survivorship between generations was observed, especially involving the first generation where survivorship was low. This variation could be due to the insects themselves or to our methods. For mass rearing purposes, efforts should be directed toward minimizing this variation.

It is more efficient to rear DBM on artificial diet than on rape seedlings. We calculated that ca. 100,000 DBM can be produced on artificial diet for ca. $60 in supplies, 40 hours of labor, and 1 m^3 space, compared with rearing on rape seedlings which would require ca. $20 in supplies, 200 hours of labor, and 12 m^3 space. Artificial diet-reared insects take longer to develop, weigh more, and lay more eggs. However, the increase in susceptibility to insecticides for artificial diet-reared DBM suggests caution when interpreting the data on leaf dip insecticide assays when using artificial diet-reared populations. These differences in susceptibility may be due to several factors including induction of detoxifying enzymes from a medium, genetic effects within the two DBM populations, or simply DBM not easily adapting to feeding on insecticide treated cabbage leaves (although no increases in mortality were seen on untreated disks). In a preliminary test to determine the duration of the influence of diet on insecticide susceptibility, we noted that DBM reared on artificial diet and switched over to rape seedlings during the second instar and then tested on cabbage leaf disks in the third instar were as susceptible as those reared on artificial diet for three instars (unpublished data). While the loss of insecticide resistance may possibly be eliminated by transferring artificial diet-reared insects to the test substrate earlier, we suggest that rearing them on rape seedlings when originally taken from the field is a safer method for three reasons. First, the lower larval survivorship in the first generation on artificial diet increased the time required to obtain enough insects to run a bioassay. Second, our artificial diet-reared DBM were more susceptible to insecticides than rape seedling-reared insects. Third, the sharp decline in LC_{50} values from the first to the sixth generation (e.g. a 73 fold decline for Lannate on diet vs rape seedlings), indicates it is important to test populations soon after they are obtained from the field. This sharp decline in resistance of DBM after several generations of rearing in the laboratory has also been documented by Sun et al. (1986).

Our results indicate the feasibility of mass rearing DBM on artificial diet and then transferring them to cabbage plants for host plant resistance (HPR) studies. Considering the low cost and ease of rearing DBM on artificial diet and the need for large numbers of DBM when running large scale host plant resistance trials on

cole crops, we recommend mass rearing DBM on artificial diet instead of rape seedlings for HPR studies. Rearing DBM on artificial diet may also be the method of choice for mass rearing of DBM for sterile insect technique programs or mass rearing of DBM parasitoids, providing studies are done to show that artificial diet-reared sterile DBM and DBM parasitoids are competitive with wild populations.

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Appendix 1. Rearing methods for artificial wheat germ-based artificial diet used for DBM, modified from Biever and Boldt (1971).

Ingredients	Amount
Mix & bring to rolling boil	
Water	3000 ml
Agar	96 g
Add and put in blender and blend for 1 minute	
Casein	126 g
Sucrose	135 g
Wheat Germ (Raw) finely ground	175 g
Add and put in blender and blend for 1 minute	
Salt mix (W)	36 g
Alphacel	25 g
Potassium Sorbate	4.0 g
Methyl-P	5.4 g
Add and put in blender and blend for 2 minutes	
Linseed Oil (Raw)	26 ml
Vitamin Mix (#26862 Hoffman-LaRoche)	36 g
Aueromycin (14% active)	4 g
Tenox (propyl-gallate)	0.8 g
KOH 45% (131 g plus 250 ml water)	9 ml
Formalin 40%	3 ml
Benlate 50 WP	3 g

The above ingredients make approximately 1 gallon of artificial diet. One gallon of artificial diet fills approx. 40 cups.

1. Egg sheets are placed into 16 oz styrofoam food cups (poured ½ inch deep with media). Cups are sealed tightly with lids to prevent larvae from escaping. Approximately 300 eggs are placed in each cup. Egg mortality of 30% will still result in app. 200 larvae/cup. Cups are kept at a constant 27° C. After 10 - 11 days, larvae will pupate around upper portion of cup and on lid. Using a razor blade, we remove upper 1 inch of cup with lid. Fifteen to 25 are set up in oviposition cages to allow for adult emergence.

2. Adults are kept in oviposition cages at 25 - 26° C and 50% RH. Cages are screened on all sides and top, one side is set up with a cloth sleeve for access. A 10% sucrose water solution, with 1.5 ml yellow food coloring, is provided as adult nutrition. Sucrose solution is placed in 250 ml flasks with cotton dental wicks inserted for adults to feed from.

3. Eggs are collected on aluminum foil strips. Aluminum sheets are cut in strips 30 cm long and 6 cm wide. Each is folded in half and crinkled (crinkling forms grooves in which they prefer to lay eggs) or, using a probe, fine parallel grooves are made in which they will lay their eggs. Strips are dipped into autoclaved cabbage juice (blend 65 g cabbage leaf material in 500 ml H_2O). Strips are dried and placed on edge in the cages. Eggs are collected every 24 hrs. Oviposition is greatest if adults are not subjected to light. After oviposition, strips are sterilized in 10% formalin for 15 min., then rinsed in a bath of running tap water for 60 min. Eggs can be held in plastic bags at 8° - 10°C until needed, and will hatch at this temperature in approximately 10 - 12 days.