

# Development and Consumption Rates For Lepidopterous Larvae as Influenced by Host-Plant and Temperature

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## Abstract

*Several laboratory studies were conducted to examine the relationships between larval developmental and foliage consumption. Neonate lepidopterous larvae were placed in controlled environment growth chambers to measure the time required to complete larval growth, and comparative foliage consumption at constant temperatures. Differences in developmental times and degree day estimates between beet armyworm and cabbage looper varied significantly with temperatures and host plant. In general, cabbage looper feeding on lettuce completed larval development at a more rapid rate and consumed more foliage than any other species-host combination. In one study, the time required to complete larval development between CL and BAW varied less than one day, but the difference in foliage consumed between the two hosts varied almost 40%. Results from these studies may provide information important for understanding the damage potential of lepidopterous larvae and optimizing use patterns for new compounds that will be integrated into management programs for leafy vegetables*

## Introduction

The lepidopterous larvae complex, consisting of beet armyworm (BAW), cabbage looper (CL), tobacco budworm and corn earworm (CE), are major pests on leafy vegetables in Arizona and annually requires intensive chemical management to prevent losses in yield and quality. Although, the biology of these species have been extensively investigated, there is no specific information describing biological development on crops such as lettuce and broccoli. The need for this information has become increasingly important as new chemical alternatives are developed for managing lepidopterous larvae.

The new insecticide active ingredients possess novel modes of action and activity that are quite different from the older chemistries that are primarily fast-acting, contact neurotoxins. For example, the new insect growth regulator tebufenozide, requires ingestion for toxicity and results in delayed mortality. Consequently, ingestion of the active ingredients via leaf consumption may influence both larval development and mortality. In contrast, compounds such as spinosad and emamectin benzoate, are neurotoxic and possess translaminar activity, that cause more rapid mortality and cessation of feeding. An understanding of the developmental rate and age structure relative to larval size may be helpful in evaluating the performance of many of these novel compounds.

Because most of the new compounds are highly efficacious against large, late instar larvae (as compared with methomyl, endosulfan, etc.) application timing becomes less critical to achieve field control. This raises an important question however. If growers wait to control late instar larvae when they are larger in size, how much additional leaf feeding (damage) occurs on plants? Thus, an understanding of the relationships between larval developmental rates, larval size relative to age, and foliage consumption through time may provide information important for optimizing use patterns for new compounds that will be integrated into insect management programs for leafy vegetables.

## Materials and Methods

*Study Insects:* Lepidopterous larvae used in this study were obtained from two sources. The first source of larvae was obtained from the USDA-ARS, Western Cotton Research Laboratory (WCRL), Phoenix, Arizona. Newly deposited eggs placed on paper towels were shipped immediately to Yuma and held at room temperature until hatching. The

second source of larvae consisted of a field population established from eggs collected on or near the Yuma Agricultural Center from lettuce or cole crops in February 1998.

*Developmental Rate Studies:* Three separate studies using the same experimental procedures were conducted to measure the amount of time required by larvae to complete development (larva to pupa). All tests were conducted in our laboratory at the Yuma Agricultural Center. The first study was conducted in December, 1998 using a population of BAW, CL, and CE larvae at 24 and 30°C obtained from the USDA and designated WCRL-I. The second study was conducted in February 1999, using a population of BAW and CL held at 24 and 30°C obtained from field collected eggs designated YAC. A third study was conducted in April 1999 from a laboratory population of BAW, and CL larvae held 30°C obtained from the USDA and designated WCRL-II.

Two environmental-controlled growth chambers were set at the above temperatures with lighting set at 12:12 hrs L:D at 24 °C , and 14: 10 hrs L:D at 30°C. No attempt was made to control humidity. The lettuce and broccoli used as food sources during these studies were obtained from untreated field plots. An attempt was made to standardize leaf age and condition. Whenever possible, younger leaves were collected from pre-heading plants, and the variety and freshness of lettuce-broccoli foliage was consistent throughout the study. The foliage was washed, allowed to air dry, and then cut into circular leaf disks approximately 60 mm in diameter.

Larvae used for these studies were placed singly into a petri dish (80 by 20 mm) containing a fresh leaf disk of lettuce within 3 hr after hatching. Each treatment contained 20 larvae, but the number of larvae that completed development varied. Mortality in the dishes was due primarily to larvae escaping petri dishes (53%), mishandling while taking measurements (27%) or of unknown causes (10%). Each dish was checked at 12 hr intervals to determine larval development. The length (mm) of each larva in a natural feeding state was measured under a dissecting scope using an ocular micrometer. Fresh leaf disks were replaced each 24 hr period. Larval instars were determined by the size of the head and by the presence of the old head capsule, which was removed from the Petri dish. Larval development was considered complete upon the presence of pupae. In the first study, pupa-adult development was measured by placing pupae in separate Petri dishes with moistened filter paper and considered complete upon emergence of moths.. Mean larval development was analyzed either as a one-way ANOVA or paired t-test. Degree day (DD<sub>55</sub>) estimates, using a base temperature of 12.5 °C, were estimated using a single sine curve method.

*Consumption Studies:* Two separate studies were conducted to measure comparative feeding rates of BAW and CL on host plants. In the first study, a population of neonate BAW and CL larvae was obtained from field-collected eggs taken from a lettuce field near Yuma. In February 1999, 25 neonate larvae of each species were randomly selected from eggs that had hatched within a 3-hr period. Each larva was transferred using a fine paint brush to a Petri dish (80 by 20 mm). Lettuce for the feeding studies was obtained and prepared similar to the development studies. A single leaf disk of lettuce was randomly selected, weighed, and placed in each Petri dish containing a single larva. After 24 hrs, all leaf disks were weighed, the Petri dishes were cleaned, and fresh, weighed lettuce disks were placed into petri dishes. Larval weights were recorded for each larva. This procedure was continued for 1<sup>st</sup> and 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar larvae and ceased at the prepupal stage. Petri dishes were held in an environmental-controlled growth chambers set at 30°C and a photoperiod of 14:10 (L:D).

Ten lettuce leaf disks of varying size were held without larvae under identical conditions as above to measure weight loss of lettuce leaf disks from dessication. A linear relationship using regression analysis was calculated between the weights of lettuce disks before the 24-hr feeding period and the weights after the 24-hr feeding period for each day's group of control leaf disks. The amount of lettuce eaten per larva per day was calculated as follows: the weight of a piece of lettuce that had been fed on was corrected for dessication using the regression equation. This value was then subtracted from the original weight of lettuce leaf disks before larval feeding. Daily consumption in grams was summed for each larva. Larval consumption and larval weights (g) for each instar and for total instars was analyzed by a paired t-test ( $P < 0.05$ ).

A second study was conducted to compare consumption of broccoli and lettuce by BAW and CL larvae. A separate cohort of larvae was obtained from the WCRL-II colony. In April 1999, 25 neonate larvae of each species were randomly selected from eggs that had hatched within a 3-hr period and placed in Petri dishes containing either broccoli or lettuce leaf disks prepared similar to the above study. Larval consumption was measured similar to the above procedure and continued for each larva until feeding ceased in the prepupal stage. Instead of measuring larval weights, the length of each larvae (mm) was measured each 24 hr period under a dissecting scope using an ocular micrometer. Daily consumption was summed for each larva. Larval consumption and larval lengths for each daily feeding period and for total feeding periods was analyzed by a paired t-tests ( $P < 0.05$ ).

*Larval age-development Models:* To gain a better understanding of the relationship between larvae size and age, quadratic regression analyses were conducted. Data used for these modeling purposes were taken from the measurements made in the developmental rate studies ( $n=40$ , BAW at 24 °C ;  $n=58$ , BAW at 30 °C ;  $n=37$ , CL at 24 °C ;  $n=59$ , CL at 30 °C). Daily larva length (mm) were regressed against the age of the larva when the measurement was made. The independent variable was larval age in days and the dependant variable was larval size (mm length). The data were analyzed with regression analysis using General Linear Models Procedure of SAS. Both linear and quadratic models were generated and compared. Because of better fits, quadratic relationships were used for this report.

## Results and Discussion

Developmental times and DD<sub>55</sub> estimates differed among BAW, CL, and CE at both temperatures regimes (Table 1). As expected, completion of larval development required more time at 24 °C, but DD<sub>55</sub> estimates were similar. Regardless of temperature, CE required a longer period of time to complete development compared with the other two species. Larva- pupa development differed significantly among the three lepidopterous species, regardless of temperature regime.

Differences in developmental times and DD<sub>55</sub> estimates between BAW and CL varied with temperatures and host plant. In the first study (WCRL-I), larval development was significantly more rapid for CL at 24 °C, but did not differ at 30°C (Table 1). Differences in developmental time were not observed between the YAC cohort of BAW and CL at either temperature (Table 2). A similar response was estimated from the third study (WCRL-II) where BAW and CL developmental times on lettuce were not significantly different (Table 3). However, host plants did significantly effect mean developmental time between species. Furthermore, larval development on lettuce was completed more rapidly on lettuce than on broccoli for both species, particularly for BAW. Differences in developmental times were most evident during the late larval instars, where BAW feeding on broccoli required a significantly longer period to complete larval development (Figure 1). These results suggest that development of BAW and CL is more favorable on lettuce than broccoli, and may be closely associated with foliage consumption.

Relative consumption rates between Lepidopterous species fed lettuce varied with larval age. In the first study using the YAC cohort, total mean consumption of lettuce was greater for CL than for BAW (Table 4). Early in larval development, lettuce consumption by 1<sup>st</sup> -2<sup>nd</sup> instar larvae was similar for both species. However, 3<sup>rd</sup> instar CL larvae consumed about 40% more foliage than BAW larvae. This difference in consumption resulted in significant differences in larval weight during this feeding period (Table 5). Overall, during larval development, the CL larvae consumed on average about 25% more lettuce foliage per day.

Foliage consumption by the two lepidopterous also varied by host plant. Mean cumulative consumption per larva per day was greatest for CL larvae provided lettuce (Figure 2), and differed significantly from all other species\*host combinations. BAW on lettuce consumed significantly more foliage than BAW on broccoli during the 7 day feeding period. Consumption of broccoli by CL was similar to BAW feeding on either host. Paired comparisons between species and hosts shows that differences in consumption occurred at distinct periods of larval development. Broccoli consumption did not differ between species during larval development, but lettuce consumption was significantly higher for 4, 5, and 6 day old CL larvae. Furthermore, consumption of lettuce by BAW was significantly greater on days 1,2, and 5 of larval development and CL consumed more lettuce foliage than broccoli at days 1,2, 5, 6, and 7. Similar to the first study, larval size differed between species. However, larval length was similar through time for CL, regardless of host. This was not the case for BAW where larval length differed significantly between host plant.

One of the interesting results of this study was that cabbage looper larvae developed at a slightly greater rate on lettuce than broccoli (Table 3). However, CL consumed a significantly greater amount of lettuce to achieve equal larval size during development (Figure 2). Time of larval development varied less than one day, but the difference in foliage consumed between the two host varied almost 40%. Differences in nutritional value for between lettuce and broccoli have been documented. This suggests that because lettuce is comprised primarily of water and cellulose, that greater consumption of lettuce would be required to satisfy nutritional requirement for CL relative to broccoli.

The information generated from these studies begins to provide us with insight into the biological development of lepidopterous larvae on leafy vegetables. Perhaps the most practical significance is that BAW and CL larvae develop at different rates depending on temperature and host plant. Learning how larvae develop on host crops relative to their feeding rates may help us understand their damage potential relative to a particular quality standard or management practice. This is important when one considers how new chemicals, with varying routes of activity, or new

management tactics may potentially be employed for control of BAW or CL in lettuce and cole crops.

The models shown in Figures 3 and 4 represent the curvilinear relationships estimated between larval size, and larval age. The models differ between lepidopterous species and temperatures consistent with our studies. We can use these models as an indication of relative differences in larvae size at each temperature. The value of these models is to allow for the prediction of larval age based on size. This can be important when evaluating larval population structure following a control measure. Ultimately, we would like to use these models for predicting ages of larvae via field measurements, but similar studies need to be conducted that incorporate fluctuating temperatures which more closely reflect field conditions.

**Table 1. Developmental times and degree day estimates for a laboratory-reared population of Lepidopterous larvae (WCRL I) and pupae held on lettuce at constant temperatures.**

Species	Temp (°C)	n	Mean Developmental Time			
			Days ( $\pm$ SD)		Degree days (DD <sub>55</sub> )	
			Larva-pupa	Larva -Adult	Larva-Pupa	Larva-Adult
Beet armyworm	24	20	11.1 $\pm$ 0.6 b	19.1 $\pm$ 1.6 b	277.2 b	382.2 b
Cabbage looper	24	17	10.4 $\pm$ 0.5 c	17.2 $\pm$ 0.7 c	261.1 c	344.4 c
Corn earworm	24	19	13.3 $\pm$ 0.7 a	27.6 $\pm$ 1.0 a	323.3 a	551.1 a
Beet armyworm	30	20	7.9 $\pm$ 0.8 b	13.9 $\pm$ 0.3 b	236.7 b	416.7 b
Cabbage looper	30	20	7.3 $\pm$ 0.5 b	12.9 $\pm$ 0.6 c	221.0 b	386.7 c
Corn earworm	30	18	10.8 $\pm$ 0.4 a	20.0 $\pm$ 0.8 a	333.0 a	600.0 a

Means  $\pm$  standard deviation followed by the same letter are not significantly different, (ANOVA, LSD<sub>0.05</sub>)

**Table 2. Developmental times and degree day estimates for a field collected population of beet armyworm, and cabbage looper larvae (YAC) held on lettuce at constant temperatures.**

Species	Temp (°C)	<i>n</i>	Mean Developmental Time (Larva-pupa)	
			Days $\pm$ SD	Degree days (DD <sub>55</sub> )
Beet armyworm	24	20	10.7 $\pm$ 0.4	229.9
Cabbage looper	24	20	10.2 $\pm$ 0.3	255.1
Beet armyworm	30	18	7.6 $\pm$ 0.5	226.6
Cabbage looper	30	19	7.2 $\pm$ 0.5	216.3

**Table 3. Relative developmental time from larva - pupa for a laboratory-reared beet armyworm and cabbage looper (WCRL II) on Lettuce and Broccoli at 30 °C.**

Species	Mean Developmental Time (Days $\pm$ SD)		<i>Pr</i> > <i>t</i>
	Broccoli	Lettuce	
Beet armyworm	9.8 $\pm$ 0.9	7.6 $\pm$ 0.5	0.0001
Cabbage looper	7.9 $\pm$ 0.8	7.2 $\pm$ 0.4	0.04
<i>Pr</i> > <i>t</i>	0.003	0.16	

**Table 4. Lettuce consumption by cabbage looper and beet armyworm during distinct larval feeding periods**

Species	<i>n</i>	Mean consumption (g)				Larval mean (g/day )
		1 <sup>st</sup> - 2 <sup>nd</sup> instar larvae	3 <sup>rd</sup> instar larvae	4 <sup>th</sup> instar larvae	Total	
Beet armyworm	25	0.67	0.76	0.51	1.95	0.27
Cabbage looper	23	0.75	1.31	0.56	2.67	0.38
	<i>Pr &gt; t</i>	0.31	0.0002	0.76	0.007	

**Table 5. Larval growth (wt) by cabbage looper and beet armyworm during distinct larval feeding periods on Lettuce.**

Species	<i>n</i>	Larval wt (g)			Larval mean. (g/day )
		1 <sup>st</sup> & 2 <sup>nd</sup> instar larvae	3 <sup>rd</sup> instar larvae	4 <sup>th</sup> instar larvae	
Beet armyworm	25	0.043	0.220	0.268	0.045
Cabbage looper	23	0.047	0.314	0.361	0.060
	<i>Pr &gt; t</i>	0.59	0.001	0.01	0.02

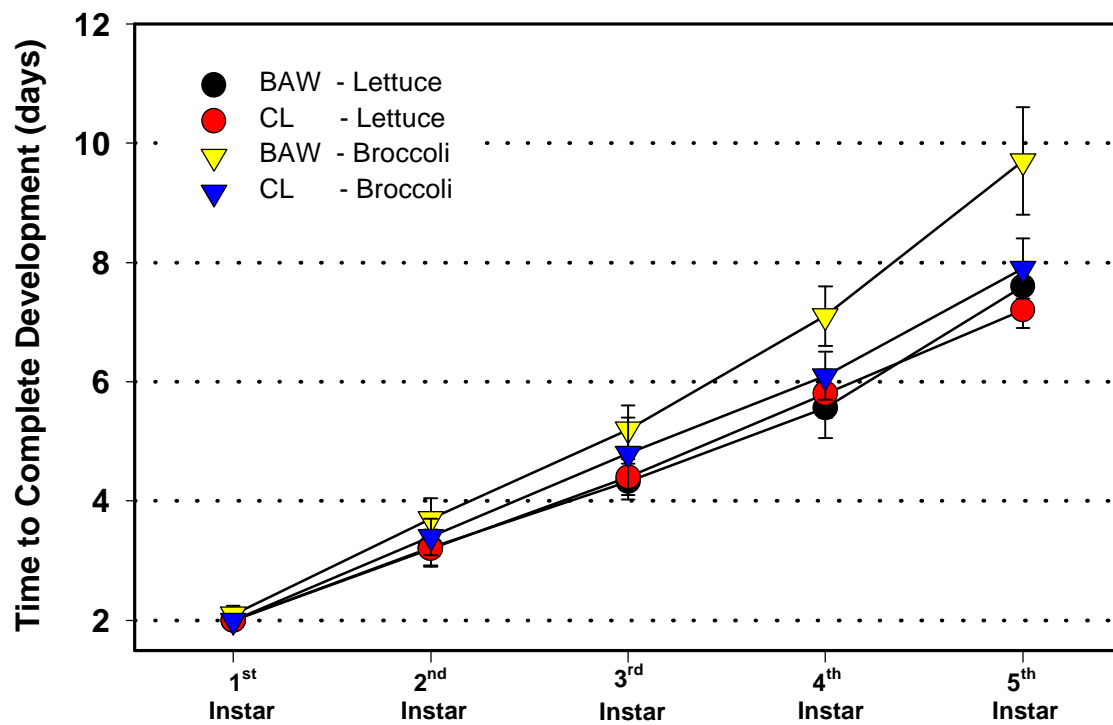
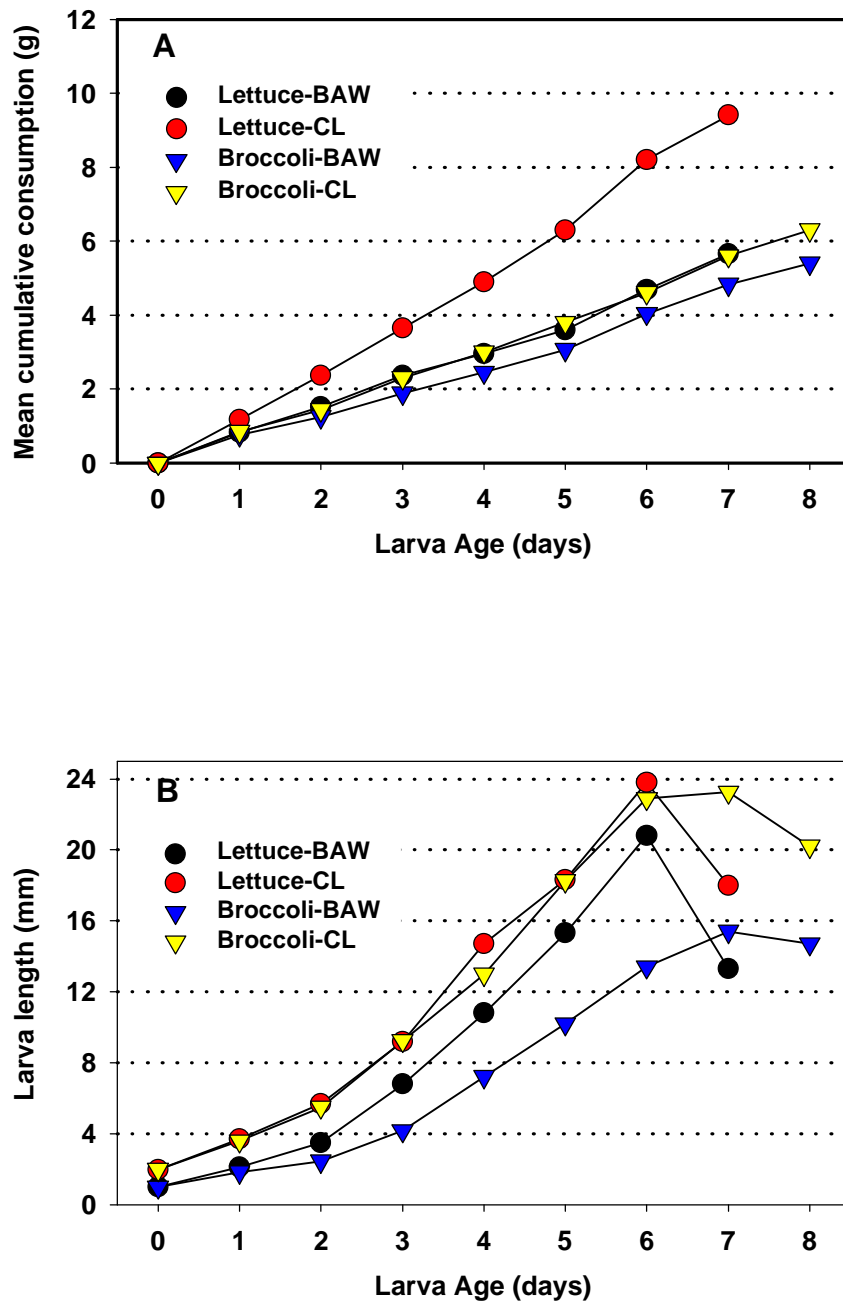


Figure 1. Developmental times for Beet Armyworm and Cabbage looper feeding on Lettuce and Broccoli at 30 °C



**Figure 2. Foliage Consumption (A) and Larvae Growth (B) of Beet armyworm and Cabbage Looper as Influence by Host Plant at 30 °C.**



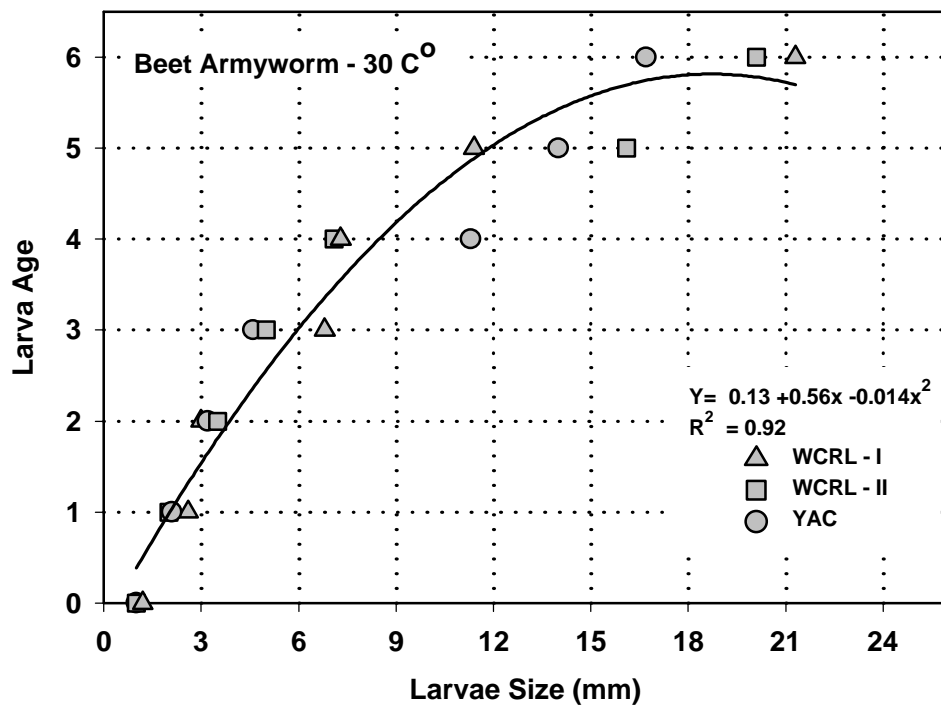
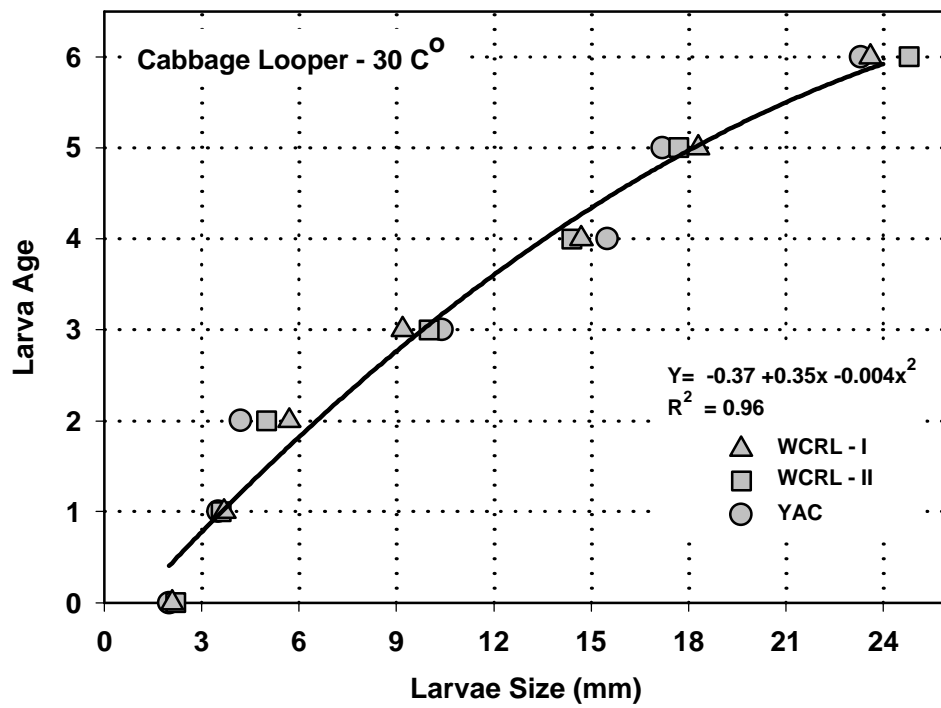


Figure 3. Cabbage Looper and Beet Armyworm Age-Development Model on Lettuce at 30 C°

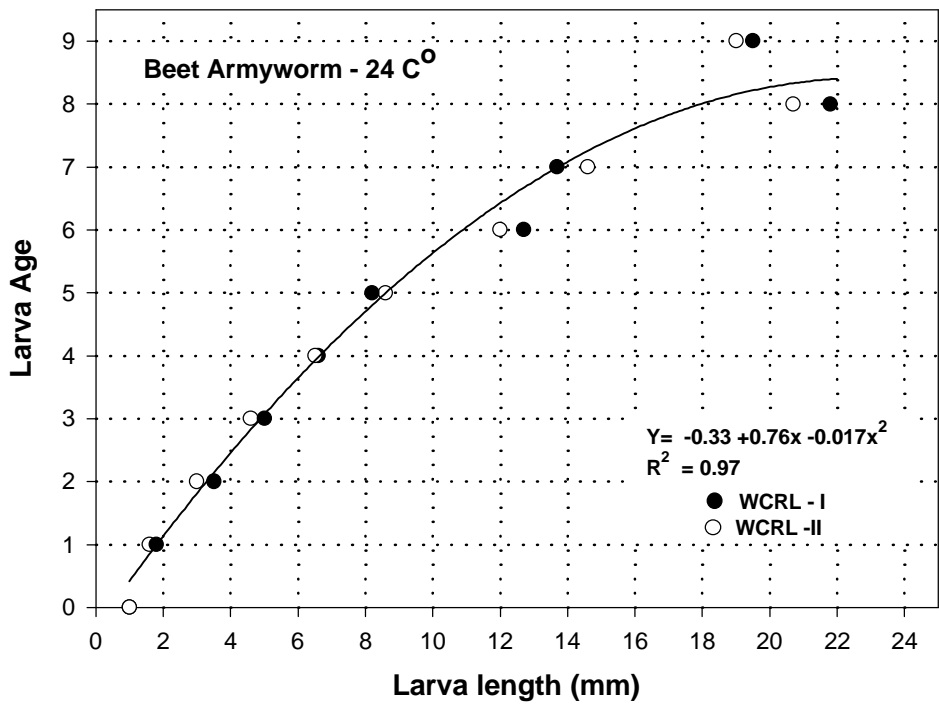
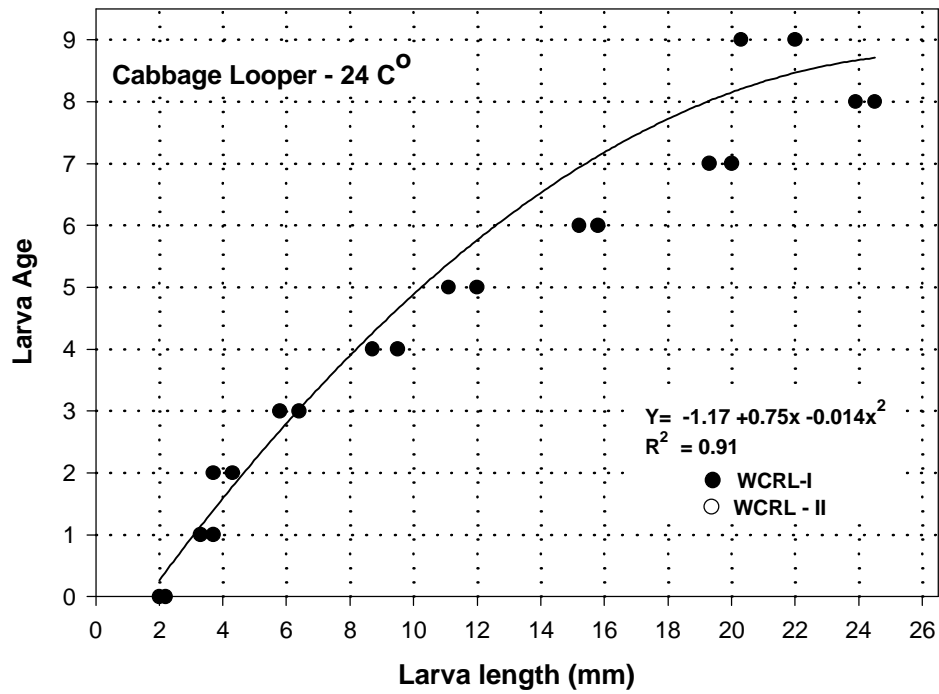


Figure 4. Cabbage Looper and Beet Armyworm Age-Development Model on Lettuce at 24 C°