Insecticide Resistance in Codling Moth: Resistance to New Selective Chemistries and Potential Changes in Developmental Rates

Stephen C. Welter and Frances Cave
Department of Environmental Science, Policy, and Management
University of California, Berkeley, CA

Studies have indicated that codling moths resistant from lab selections to diflubenzuron (Dimilin) or deltamethrin (pyrethroid) exhibited significant increases in development times (number of degree-days to complete a generation) (Boivin et al, 2003). The resistant lines had slower developmental rates compared to susceptible populations. These data may have potential implications for the 2 peaks of codling moth now commonly observed in California pear orchards from the overwintering flight. The development of a 1A and 1B peak has coincided with increasing resistance in California and is now being reported observed in many orchards in Washington, which has been slower to evolve resistance.

If two genotypes in codling moth exist that differ in their degree-day requirements, this variability would be expected to limit the utility of our degree-day models or potentially to force the alteration of the parameters. Secondly, the potential exists for differing levels of resistance at the different peaks of a generation with the 1B peak perhaps having greater resistance levels (and hence slower developmental rates).

Our goal was to sample both 1A and 1B peaks from each of several sites in the delta for two types of assays: 1) measure current levels of Guthion resistance, and 2) evaluate the development time of progeny developing from each of the peaks. In addition, we are endeavoring to 3) evaluate the compounds Intrepid and Novaluron with resistant and susceptible codling moths.

Materials and Methods

Guthion Resistance Assays. Guthion resistance assays were conducted for three delta orchards at the 1A and 1B codling moth flight periods. Dose mortality data were compared between peaks within each of the delta sites. All three delta sites were treated with Guthion shortly after our 1A samples. In addition, another resistant population was sampled from a conventional apple orchard near Gridley, CA.

Assays were conducted with using standard protocols developed over past years. Pheromone traps using liners coated with approximately 1.5 ml Tanglefoot were placed in Trécé Delta VI traps. Traps were baited with a codlemone lure (1X if a conventional site or 10X if a pheromone treated site). Approximately 120 traps would be placed in a site before dusk and collected at daybreak the following morning. Liners with moths were removed from traps, stacked in an ice chest to keep moths cool, and returned to our Berkeley lab where they were treated with a dose series of pesticide in order to establish a dose response line. The pesticide was delivered to individual moths by application of a 1 microliter aliquot to the ventral posterior abdomen of each moth. Treated moths were then held 48 hours at 15^{o}C.

Mortality was assessed by brushing the moth with a fine camel-hair brush; moths that failed to respond with vigorous leg movement were scored as dead. Probit regression lines and LC50 values were estimated using the probit option of POLO (LeOra Software, Berkeley, CA). The lethal concentration ratio test (LCR) was used to compare regression lines for significant differences.

Development Time for Progeny of Moths from the Overwintering Generation in the Sacramento Delta

Female collections. Studies of resistance in codling moth have been plagued by the fact that resistance is difficult to maintain in lab colonies because organophosphate resistant females lay fewer eggs than susceptible females. The hope was that collections of live moths in the field would allow for either direct assays on egg or newly hatching larvae. Codling moth will approach and orient to an ultraviolet light source. We used this behavior to attempt night collections of females (and males) beginning late March and continuing through both flights into late May. The basic technique used was as follows. A site (or sites) in the orchard was selected that presented a less densely canopied area so that the visual cue of the UV light would have visibility from a greater distance. A white bed sheet was suspended between trees and two UV lights running on 12v batteries were hung at the top of the sheet. Typically, two sheets were hung in close proximity at a "station", one suspended between rows and one within a row. A DA lure was placed near the top of the sheet to provide an additional orientation cue. As codling moths flew into the sheet, they were immediately collected individually into 1 ounce plastic vials. As the moths would generally not rest on or remain at the sheets for more than a brief contact, each station had to be constantly monitored during the evening. UV lights would be turned on at sunset and collecting efforts terminated when an evening flight stopped or temperatures dropped below the flight threshold. Typically this meant arriving in the orchards prior to dusk and monitoring until late in the evening. Vials were placed in an ice chest for transport to the Berkeley lab at the end of each night. Collections were made from three sites in the Sacramento delta that were also sampled for Guthion resistance. While this approach was presumed to be fairly easy, this effort proved otherwise. An approximate total number of 100 "man-nights" were invested into this effort to collect live moths over 25 night collecting trips. Female codling moths were rarely caught on many nights with cool temperatures. The literature has suggested that females will adjust their calling behavior in response to cool evenings by emitting their pheromones before dusk. Unfortunately, this means that the blacklights, which are not effective until nightfall, may have been severely limited in terms of efficacy for attracting females during the spring.

Larval-Pupal development time measurements. All moths from a site were sexed the morning after collection and females and males were placed in an oviposition cage in the lab. Typically, newly collected "wild" moths resist mating or egg laying in lab conditions.

To try to overcome these obstacles, the cages were placed in a window so that moths were exposed to natural light conditions. In addition, the waxed paper egg sheet would be treated with (organic) apple residues and cut apples would be placed next to the cages to stimulate egg laying. Egg sheets were collected and held at approximately 80^{o}F to develop. Once hatch began, larvae were transferred individually to diet cups twice a day until hatch was complete. Cups were labeled for parent source and transfer date and time and then held at approximately 80^{o}F and a 16:8 light:dark cycle. Temperature in the development room was monitored by a HOBO H8 data recorder programmed to read at 1 hour intervals. Prior to the first adult emergence, we began inspecting vials twice a day, to record final development time. Time and date of emergence for each vial was recorded. Degree days for larval-pupal development were calculated as the time a larva was transferred to the diet until adult emergence using the temperature data from the HOBO unit.

Development time of eggs and larvae from resistant and susceptible apple populations. In addition to the codling moth population from the Sacramento delta, we brought two apple populations into the lab in summer and fall of 2004. The resistant apple population was collected in August by UV light sampling in the conventional orchard near Gridley, CA. A presumed susceptible apple population was started in October 2004 from larvae collected from dropped fruit in an organic apple orchard near Philo in the Anderson Valley of California. This orchard has been in an organic program for about 20 years. While we have not been able to conduct a resistance bioassay to date, we anticipate this to be a susceptible population based on its history and location and resistance data from a nearby abandoned orchard. Development time from egg hatch to adult emergence was calculated by the methods described above for the delta populations. In addition, we compared the development time of eggs for the two apple populations. Egg sheets were collected on a daily basis for four days. Twenty-five eggs were marked on each sheet for a total of 100 eggs per apple population. These were held in separate rearing rooms with temperatures recorded on an hourly basis using HOBO data recorders units. Eggs were inspected several times a day for hatch and degree days for development estimated.

Egg and larval response to Novaluron and Intrepid. Because of the difficulty collecting field populations for direct assays, these trials will be conducted with the newly established resistant and susceptible populations collected in late 2004. As of December 2004, these colonies are beginning to provide the numbers of specimens needed for the assays. We anticipate completion of this work by early February 2005 following assays to confirm resistance and susceptibility status with Guthion.

Results

Guthion Assays. Assays in three delta orchards were successfully conducted for both 1A and 1B flights. We were able to trap male moths during flights at 200-250 DD (1A) and 600-770 DD (1B). The resistant apple population near Gridley was assayed later in the season, in early August.

For the three delta populations, resistance levels in the 1A flight ranged from 0.38 to 0.57 micrograms per moth while those from the 1B peak ranged from 0.27 to 0.55 µgm per moth (Figure 1). No distinct pattern of differences in resistance levels was observed between the 1A and 1B peaks of each site. However, all sites evaluated are resistant to Guthion compared to historical LC_{50} values for susceptible orchards at ca. 0.06 micrograms per moth.

A significant difference in response between peaks was indicated by the LCR test only at the Sutter Island site where LC_{50} values for 1A and 1B peaks were measured at 0.379 (±0.12) and 0.547 (±0.18) µgm per moth, respectively. Differences in 1A and 1B flights at the other two sites were not significant. We measured LC_{50} values of 0.384 (±0.16) and 0.272 (±0.17) µgm per moth at the Randall Island site, and 0.571 (±0.14) and 0.545 (±0.16) µgm per moth at the Courtland site for 1A and 1B flights, respectively. The conventional apple site we assayed yielded the highest LC_{50} value this lab has measured to date. The August assay indicated a LC_{50} value of 0.985 (±0.216) µgm per moth. The increased levels of resistance with LC_{50}values of 0.985 µgm per moth in the apple orchard are considerably higher than levels of resistance first noted in 1989 (0.22 µgm per moth). The pattern of Guthion resistance has remained relatively consistent with a slow, gradual increase in resistance levels, but without the rapid rises often seen in other pest species. Anecdotal evidence from S. Africa has suggested that serious control failure issues were correlated with resistance levels at LC_{50} values of ca. 1.2 µgm per moth.

The potential for strong differences in the codling moth that emerged at different times in the orchard was not evident in our data. As such, there does not appear to be any need at this time to change control practices to address potential differences in resistance. While variability clearly exists between orchards, management decisions

Larval-Pupal development times. As stated above, adult female collections proved far more difficult than anticipated. A minimum of 25 night collection trips were made, each using the efforts of from two to six people. The typical scenario was that temperatures would rapidly drop after sunset and little, if any, codling moth activity would be seen at the UV lights. Some collections of adults brought into the lab failed to lay eggs. When moths were flying to the lights, males tended to outnumber females by a ratio from about 3:1 to 2:1. We did succeed at collecting adults that reproduced from all three sites that we assayed for Guthion resistance. However, we were not successful at collecting the populations at the same time as the resistance assays. None of our attempts at collecting during the early part of the 1A flight were successful in providing larvae for the development assay. The number of females used to initiate a larval population was low for most collections and thus our results may not reflect the diversity of a population. For those samples that successfully produced larvae for the development time trial, adult collection time (DD) and numbers for each delta site are shown in Table 1.

Table 1. Founding numbers of codling moth adults (females (f) and males (m)) from night collection samples in the Sacramento delta that yielded eggs and larvae.

	Site		
Degree Days	Randall Island	Courtland	Sutter Island
466			5 f + 20 m
490		25 f + 49 m	
507			10 f + 8 m
525	14 f + 21 m		
611		55 f + 167 m	
732	3 f + 8 m		

The night collections from the Gridley, CA conventional apple site yielded in excess of 300 moths with about 85 females. A night collection from the organic apple site in Philo was not successful; however, larvae isolated from infested fruit provided about 20 adults to initiate the colony. (Tree bands placed in the orchard have yielded several hundred additional larvae that will be added to the new colony after they break diapause.)

Egg Development Rates. Egg development time was estimated only for the organic and resistant apple colonies collected later in the season. In this preliminary trial, it appears that the resistant population exhibits a significant increase in development time by about 8% ($P<0.05$). Average time for egg hatch in the organic colony was estimated as 139 DD (SE=1.6) compared to the resistant population time of 150 DD (SE= 0.9) (Figure 2). This trial should be repeated and refined to constrain the oviposition sample to a shorter period to better estimate actual egg age and to record hatch data at regular intervals across a 24 hour cycle.

Egg Hatch to Adult Emergence. Development time from egg hatch to adult emergence in these same organic and resistant populations followed a similar trend as the eggs with slower developmental rates observed for the combination of the larval and pupal stages. However, differences in degree-days between males and females were correlated with changes in OP resistance.

Difference in development time in females from the organic and OP resistant apple orchard was greater than the differences between males from each area. Females of the Gridley population took on average 12% longer to develop than the males. The Gridley females emerged as adults on average at 1029 DD (SE=6.2) after hatch, compared to the organic population average of 918 DD (SE=13.8). Data for female progeny of moths collected in the Sacramento delta are shown in Figure 3, as well as for the apple strains. The Sutter Island data is combined into one data set, as successful adult collections were made only 2 days apart. Development time ranged from 891 to 1023 DD with no pattern emerging between earlier and later parent collections.

High variation in mean resistance levels exists between colonies and within collections on different sampling dates. The fact that some collections had few females captured on a particular date may present a problem with strong founder effects (e.g. the random selection of a few females that may have had more or less resistance than the average female in the collection had a strong effect on the average resistance level. Therefore, collections of progeny from smaller number of founding individuals (e.g. < 20) make these collections more suspect. Conversely, collections from >200 individuals should be more robust.

Development times for male larvae ranged from 870 to 966 DD from the Delta sites Again, no obvious pattern emerged to indicate slower development from moths that emerged later in the flight period (Figure 4). Development time for males from the resistant population was increased by an estimated 6% compared to the organic population. Average time to adult for males of the resistant Gridley colony was 989 DD (SE=5.48) compared to 934 DD (SE=13.3) for organic colony males. Thus, these differences are small on average and would not be expected to drive the trends.

Figure 5 shows the full range of development data for the first successful collection (April 28th) of adults from the Courtland site. Development times for each larva are plotted. The development curves within a single orchard demonstrate that males develop sooner than females, but the effects would not be very dramatic.

Conversely, if you examined the expected emergence curves against degree-days for females from the organic and OP resistant apple orchards, then the effects become more significant (Figure 6). One pitfall of using averages between populations assumes that all individuals are affected equally and that resistance levels are equal. The portion of the curve which is potentially disturbing are the end points in which a small fraction (ca. 20% or less) may have dramatically slower developmental rates compared to the average. This is best shown in Figure 6 in which 100% of the individuals from the organic orchard would be expected to have completed development while 20% of the population would have yet to emerged from the OP resistant strain. If the numbers of codling moth within an orchard are high, then this small fraction may present a serious challenge to control efforts timed at average development times if pesticide residues were insufficiently short to control this later emerging 20% of the population.

However, use of trap monitoring of males within pheromone traps would not be expected to detect this delayed emergence patterns as the males from the same collections (organic versus OP resistant apples) do not have as large of a difference (Figure 7).

These data are suggestive of potential problems yet not conclusive. The strong differences between the apple colonies is actually only one pair that would need to be verified before strong conclusions could be made. Similarly, the fact that insufficient numbers of individuals were collected in some cases due to difficulties in night trapping codling moth also suggests caution before interpreting these data very hard. At this time, it would be prudent to continue direct fruit examination for early signs of strikes by codling moth beyond expected egg hatch, especially in orchards with OP resistance or problems.

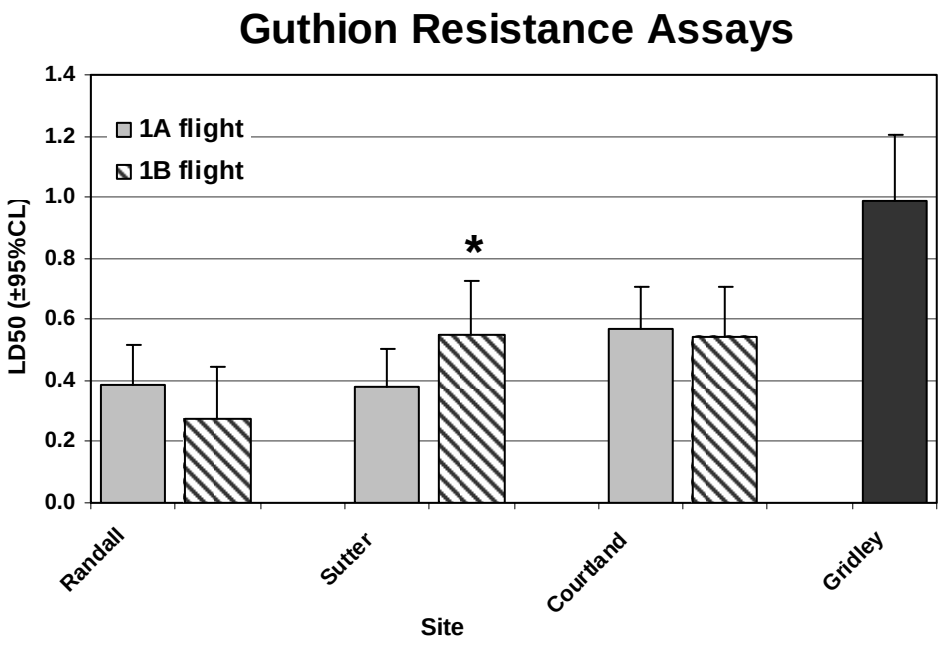

Figure 1. Guthion resistance levels (µgm per moth) of populations collected from three pear sites in the Sacramento delta at 1A and 1B peaks, 2004 and a conventional apple orchard near Gridley, CA. (*) denotes a significant difference in value between 1A and 1B peak measurement for the Sutter Island samples as determined by a LCR test.

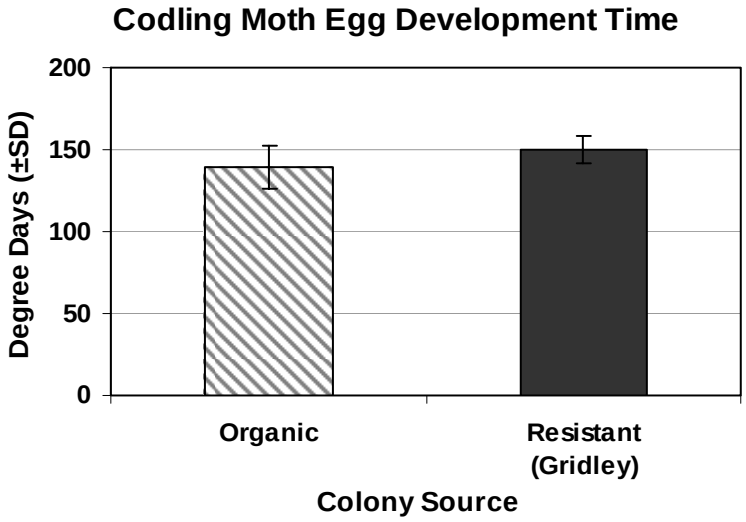

Figure 2. Estimate of development time from egg laying to hatch for two populations of codling moth collected from apples.

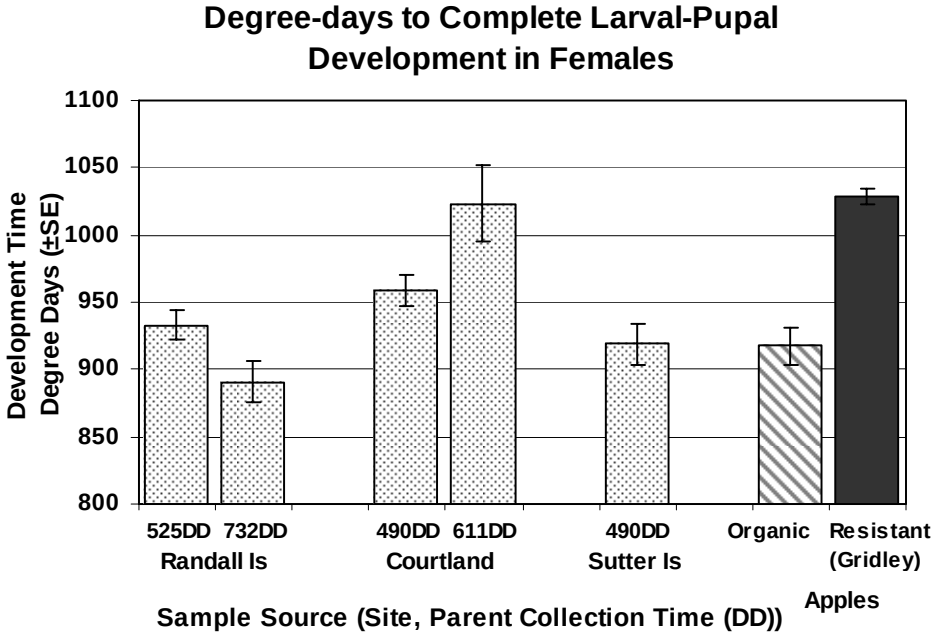

Figure 3. Number of degree days required to complete development from egg hatch to adult emergence for female codling moth for 3 pear orchards in Sacramento Delta with OP resistance and 2 apple orchards. Multiple collections from each orchard are indicated by the number of degree-days (DD) for each sample.

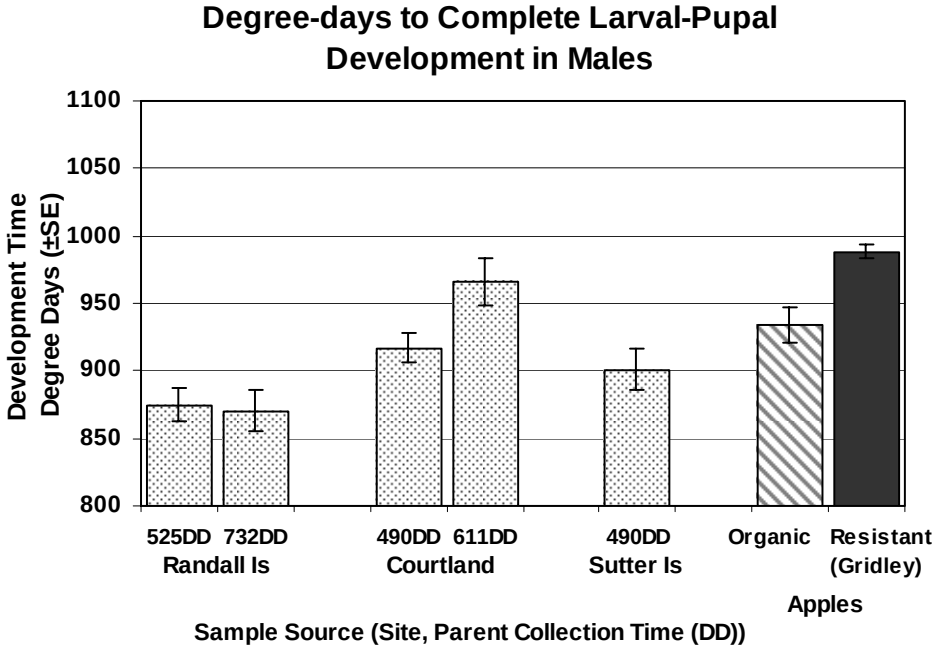

Figure 4. Number of degree days required to complete development from egg hatch to adult emergence for male codling moth for 3 pear orchards in Sacramento Delta with OP resistance and 2 apple orchards. Multiple collections from each orchard are indicated by the number of degree-days (DD) for each sample.

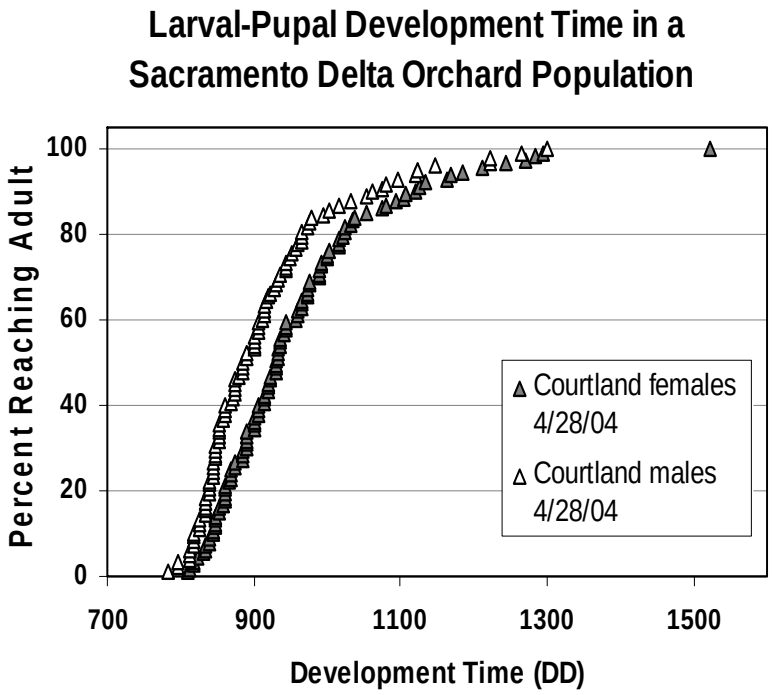

Figure 5. An example of a development curve derived from male and female progeny of codling moths collected April 28th from the Courtland area orchard. Degree-days measured from neonate larva to adult emergence.

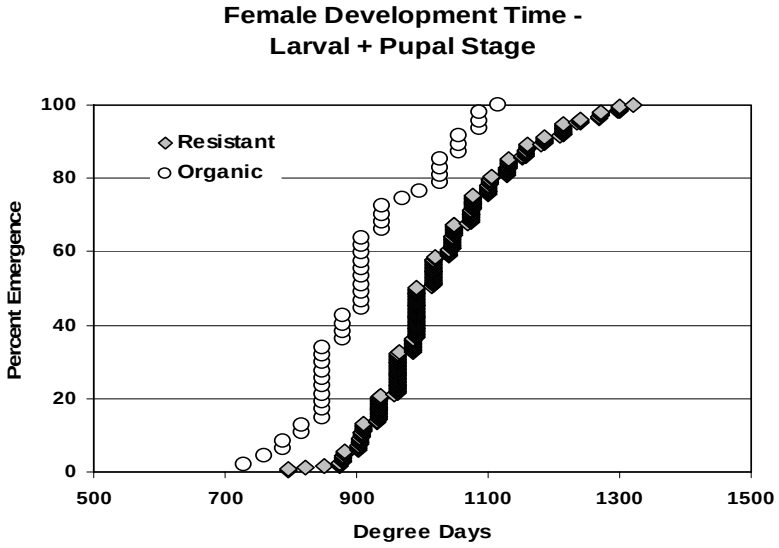

Figure 6. Adult emergence curve for females of the organic and resistant (Gridley) populations. Degree-days measured from neonate larva to adult emergence

.

Figure 7. Adult emergence curve for males of the organic and resistant (Gridley) populations. Degree-days measured from neonate larva to adult emergence.