Trade-Offs Between Insecticide Resistance and Development Time in Codling Moth

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Abstract Resistance against organophosphates in the codling moth, *Cydia pomonella*, is suggested to increase the development time between life stages. Such life history trade-offs are not uncommon, but may pose a threat to current pest management strategies. Differences in development time for susceptible and resistant populations will not only result in two distinct emergence peaks, but may also consequently force an alteration in the timing of pesticide applications. The aim of this study is to confirm a difference in development times between susceptible and resistant codling moths. A colony of each genotype was reared in a laboratory and adults were mated to produce eggs. Freshly laid eggs were stored in growth chambers at 55°, 70°, 80°, and 90°F. Lower developmental thresholds were found to be 50.41°F and 51.74°F for the susceptible and resistant colonies, respectively. Degree-days were calculated using a lower threshold of 50°F, and eggs were examined for hatch every ten degree-days. Organophosphate resistant codling moths were found to have longer egg-larva development times. The bimodal emergence pattern observed between these two colonies is of particular concern at cooler temperatures, spanning larger amounts of time between peaks. These differences imply that identifying levels of pesticide resistance in codling moth populations is an important consideration in assessing the utility of current degree-day models.

Introduction

Codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), is a major pest in apple, pear, and walnut orchards across the world, causing huge economic losses to agriculture (Bouvier *et al*. 2002). During the summer, over half of all insecticides used on apple orchards are used to control against codling moth (Brunner *et al*. 2002). Multiple generations of codling moth have been observed to attack orchards continuously for the entire duration of a harvest (Bouvier *et al*. 2002). Among the many pest management methods employed by crop-growers is the use of organophosphate insecticides.

Studies researching other forms of chemical pest management have indicated that removing organophosphates from current codling moth management programs will be difficult (Knight *et al*. 2000). Though intended for the codling moth, these insecticides are also effective at indirectly controlling many secondary pests associated with the codling moth, such as the leafroller (Walker and Welter 2001). Because of their effectiveness and their limitation of secondary pest outbreaks, organophosphate applications are widely used in California and Washington (Brunner *et al*. 2002).

Frequent application of insecticides can create selective pressures on pests, forcing allelic substitutions that lead to genotype variation and insecticide resistance (McKenzie and Batterham 1994). These anthropogenic perturbations can force a reallocation for different enzymatic activities (Boivin *et al*. 2003a). Due to the wide use of insecticides, some strains of codling moth have developed a resistance to the organophosphates. Codling moths are becoming increasingly more resistant to benzophenylureas (BPUs) such as diflubenzuron (Sauphanor *et al*. 1995, Bouvier *et al*. 2002). Selective pressures can result in cross-resistance, enabling codling moth to detoxify a variety of different chemicals, even when the mechanisms by which chemicals are toxic differ from one another (Dunley and Welter 2000).

While insecticide resistance poses a problem for pest management by making insecticides ineffective, another indirect situation may develop as a result, and could potentially pose a problem. Resistance often comes with a cost to the pest. Life history trade-offs can occur in many forms, including reduced fitness or size. Particularly, pleiotropy can result in a selective disadvantage in regard to development (Boivin *et al*. 2003a).

There is some evidence that organophosphate resistance in codling moth is correlated to a decrease in development rates (Boivin *et al*. 2001). More degree-days are required for resistant

codling moths to complete their developmental stages. The two distinct genotypes of resistant and susceptible codling moths differ not only in their susceptibility to insecticides, but also in their peak emergence times. Development plays a key role in timing of pest management (Sauphanor *et al*. 1997).

Crop growers' ability to control codling moth populations is particularly important because of the large role that codling moth plays in many economically important crops. Having multiple peaks in the development of codling moth could potentially limit the predictive capabilities of existing degree-day models. These models are of particular importance because choosing the period to apply insecticides has an effect on insecticide efficiency and the potential of pests to develop further resistance. Frequent exposure of pests to sublethal doses of an insecticide could result in a gradual decrease in susceptibility (Sauphanor *et al*. 1998). Non-overwintering populations are also observed to have slower developmental rates (Boivin *et al*. 2001), which will further limit the efficacy of degree-day models. Confirming and quantifying the difference in degree-day requirements for these two strains of codling moth will help in identifying the developmental implications of organophosphate resistance.

Methods

Adult moths were obtained from apple orchards in northern California by Welter and Cave (2004). Bioassays for a prior experiment were conducted to determine the susceptibility of moth populations to insecticide. The organophosphate-resistant and organophosphate-susceptible colonies were reared and kept in separate rooms to prevent contamination through cross-breeding. Anywhere from 15-50 adult moths from each colony were placed in cylindrical mating chambers, depending on the population sizes of the colony at the time of mating. The chambers consisted of a tube of wax paper with an approximate area of 3 ft^2 wrapped around an apparatus with wire mesh ends, with a different chamber for each of the two genotypes. The moths were given a window of 6 hours to lay and hatch eggs, coinciding with a simulated dusk on a 16L:8D light cycle. Eggs were laid on the wax paper, which was processed, recorded, and replaced with clean wax paper after the 6 hour mating session.

Each egg sheet of wax paper was examined for eggs. The eggs were circled and numbered so that each individual egg's development could be monitored over time. The egg sheet was then cut into sections of 20-30 eggs each section. A set of four replicates of the egg sheet sections were

placed in sealed boxes in one of four growth chambers, set at 55, 70, 80, and 90 °F. Temperature was recorded at two hour intervals using a HOBO H8 data recorder.

Eggs in the 70-90°F were checked for eclosion every 10 degree-days and were recorded as eclosed as soon as the visibly black heads of larvae were no longer present in the egg. The 55°F chamber was checked every 5 degree-days, or once every 24 hours. The lower developmental threshold of the codling moth has been observed to be 50°F (Howell and Neven 2000), and degree-days were calculated using this threshold. Degree-days for larval development were calculated using temperature data from the HOBO with the time that the egg sheets were entered into their chambers as the start time and the average time between observed eclosion and the previous check as the end time.

The egg-larval development stage was chosen for monitoring because an egg is self-contained and only requires heat to hatch. Howell and Neven (2000) consider the egg's narrow development time a favorable feature in selecting development stages for use in modeling phenology.

Because the resistant population was not acclimated to rearing in a laboratory environment, the sample sizes of that population were sometimes more than two times smaller than the susceptible population. Table 1 lists the sample sizes of each treatment and genotype.

Table 1. Sample sizes for the different treatments. Limited egg hatch from the resistant population prevented equal sample sizes.

Genotype	*n* for 55°F treatment	*n* for 70°F treatment	*n* for 80°F treatment	*n* for 90°F treatment
Susceptible	118	143	132	120
Resistant	39	82	98	64

Results

Eggs reared in the three warmer temperatures hatched much more quickly proportionally to eggs placed in 55°F chamber l(Fig. 1). The average egg in the 55°F hatched in 649.59 hours for the susceptible moth and 700.69 hours for the resistant moth. Across all temperatures, susceptible larvae were the first to emerge. The comparison of emergence time between these two genotypes is more apparent in measurements of thermal units, seen in Figure 3.

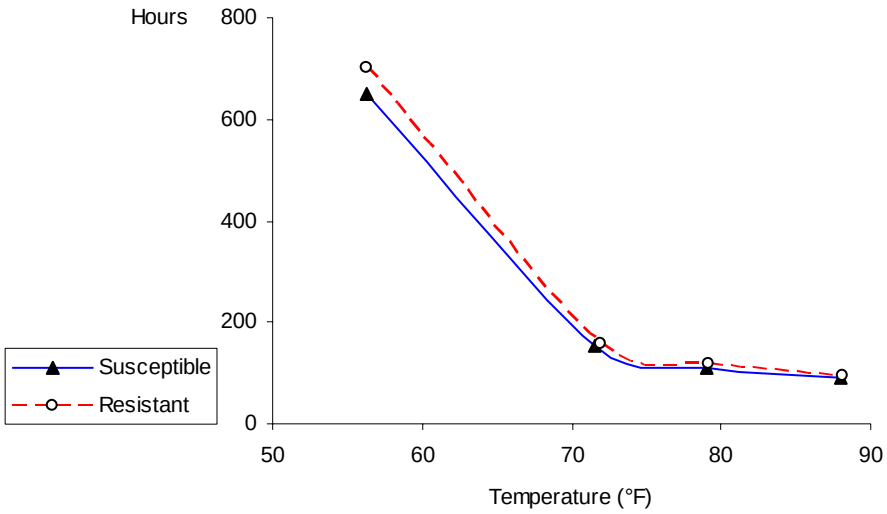

Figure 1. Average total time for egg hatch in each of the four treatments.

Total numbers of hours for average egg hatch were used to extrapolate a lower developmental threshold for codling moth. Average daily hatch rates were calculated for each temperature treatment based on the mean hour requirement, and each genotype had a linear regression model fit to it based on hatch rates l(Fig. 2). From these regression lines, the lower developmental threshold was extrapolated by solving regression equations for y=0. Based on these data, the x-intercept and lower threshold for the susceptible colony is 50.41°F and the lower threshold for the resistant colony is 51.74°F. Analysis of covariance (ANCOVA) found no difference between the two regression lines (P > 0.50)

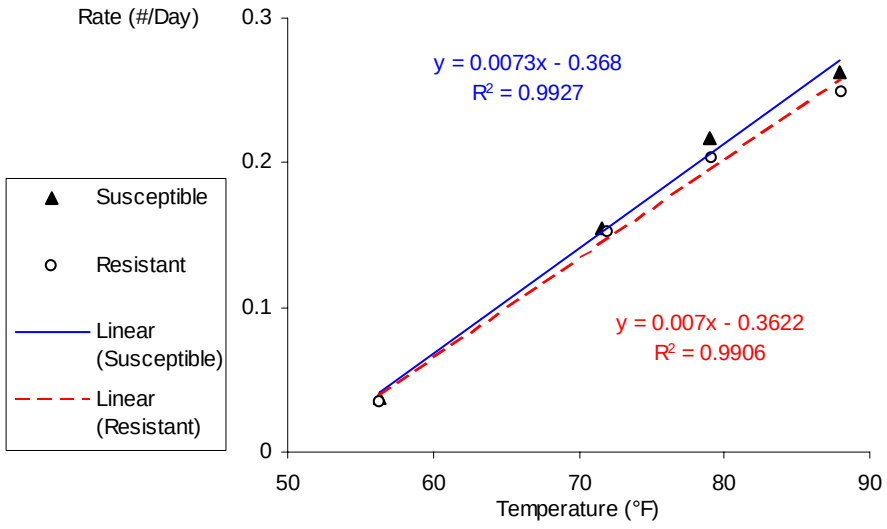

Figure 2. Rate of egg hatch in eggs per day across the four treatments with a simple linear regression through each genotype. Regression equations with R^2 values are found closest to their regression lines.

Mean development time from egg to larva varied across the different temperature treatments, with 132.39-157.87 degree-days for the susceptible colony and 142.63-186.34 degree-days for the resistant colony l(Fig. 3). Considering each treatment a set of two replicates, the resistant population consistently required more degree-days to develop than the susceptible. The smallest difference between the pairs in a treatment was 18.11 degree-days at 70°F and the largest difference was seen at 55°F with 40.18 degree-days. A paired Student's *t*-test performed on the two genotypes using the four treatments as replicates revealed a difference between all mean degree-day requirements ($P < 0.081$). Combined averages for the two populations were 143.28 degree-days for the susceptible and 157 degree-days for the resistant.

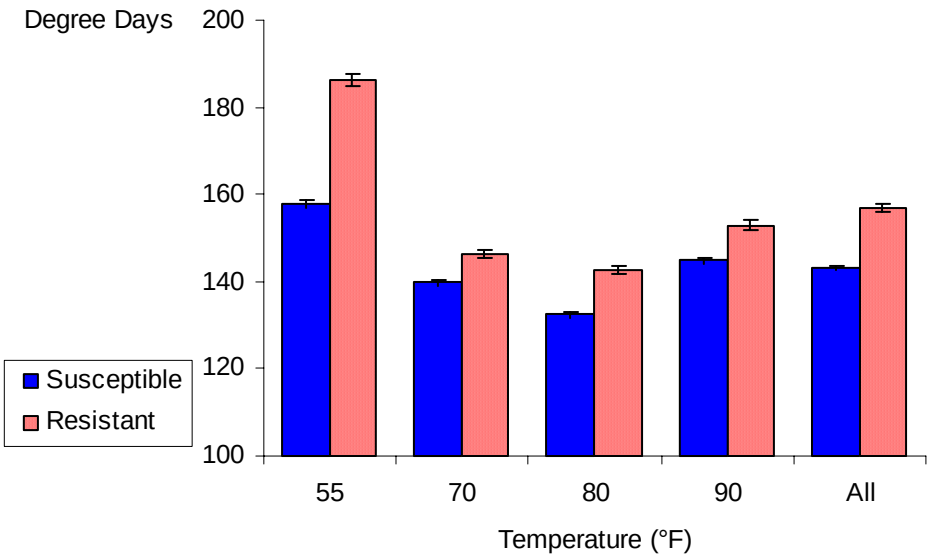

Figure 3. Mean degree-day requirements for egg-larval development across four temperature treatments with standard error bars. "All" indicates the combined average of the means.

Using the combined average as an estimate of total degree-day requirement to complete the egg-larval development stage, the resistant colony took 13.72 more degree-days to hatch than the susceptible colony. This increase of corresponds to a 9.8% increase in development time.

Resistant and susceptible eggs began hatching at the same time in the 80°F and 90°F

chamber, and susceptible eggs continued to hatch more than 8 degree-days after the resistant eggs finished at 90°F l(Fig.s 4c-d). As seen in Figure 3, these two chambers still had resistant larvae hatching slower on average.

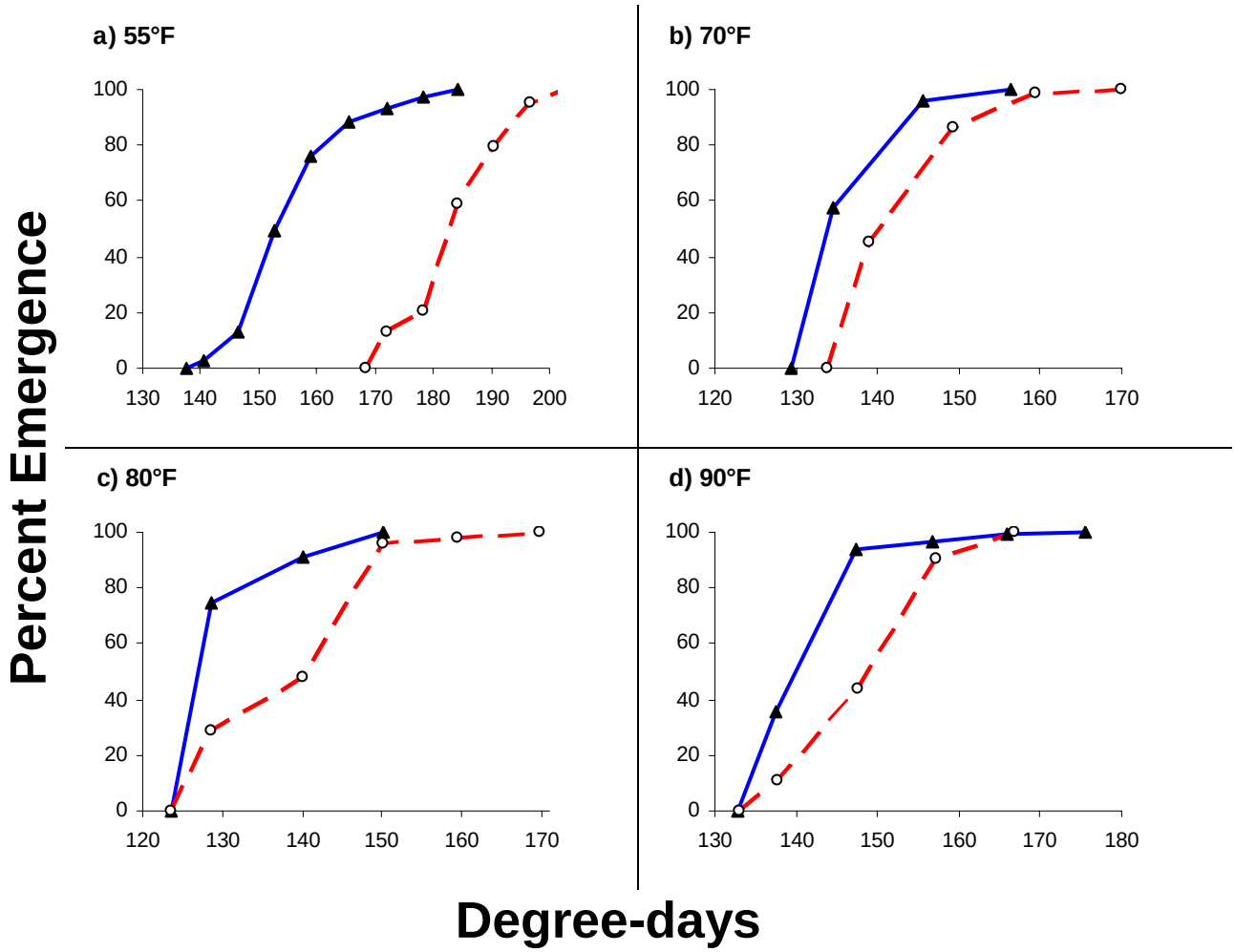

Figure 4. Emergence curves for development from egg to larva for both susceptible and resistant populations across four temperatures (a-d). Percent emergence was calculated by ranking larva in order of hatch and dividing by the sample population.

The susceptible eggs in the 55°F and 70°F chambers hatched consistently before the resistant eggs l(Fig.s 4a-b). At least 88% of the susceptible population hatched before the resistant began hatching at 55°F.

Discussion

The difference between the lower developmental thresholds for the susceptible and resistant colonies may suggest that these thresholds, like development time, differ for these genotypes due to pleiotropic interactions. However, the difference between them is not significant, and more samples and replicates would be needed to confirm that the extrapolation of the x-intercept is accurate. If these thresholds are different, then these genotypes would accumulate different degree-days, contributing to the slower development time for resistant moths.

The rate of development over temperature ranges increases similarly between the two populations. Without examining the difference in development time between different life stages, this could imply that resistant moths don't have different rates but just take longer to start developing. Assuming the rates are the same for these populations in larval-pupal and pupal-adult life stages, the difference in total development time may not be as large as it was observed in this study.

The increase of 9.8% in development time could potentially cause nearly a two-day gap in hatch in the field with an accumulation of 7 degree-days per day. The implications of this latent emergence would mean a re-evaluation of spraying time and the current degree-day models. Unless new models time sprayings for both peak emergences, this also implies that the genotypes of codling moth populations would need to be known prior to management.

Sauphanor *et al*. (1997) found that resistance in codling moth to diflubenzuron came as a result of enhanced cytochrome P450-dependent monoxygenases (MFOs) and glutathione *S*-transferases (GSTs). This results as a dose-response relationship, as resistance is an autosomal recessive trait (Bouvier *et al*. 2001). The rate of resistance can be reduced by reducing selective pressures and practicing other forms of pest management (Boivin *et al*. 2005). A study by Boivin *et al*. (2003b) found that a colony of heterogeneous moths with a frequency of resistance 0.77 decreased to 0.24 over 10 generations of living in the absence of pesticides. The use of non-chemical pest management may be a temporary solution to making insecticides as effective as they could be.

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References

Boivin, T., J.C. Bouvier, D. Beslay, and B. Sauphanor. 2003a. Phenological segregation of insecticide resistance alleles in the codling moth *Cydia pomonella* (Lepidoptera: Tortricidae): a case study of ecological divergences associated with adaptive changes in populations. Genetical Research. 81(3): 169-177.

Boivin, T., J.C. Bouvier, J. Chadoeuf, D. Beslay and B. Sauphanor. 2003b. Constraints on adaptive mutations in the codling moth *Cydia pomonella* (L.): measuring fitness trade-off and natural selection. Heredity. 90(1): 107-113.

Boivin, T., C. Chabert-d'Hières, J.C. Bouvier, D. Beslay and B. Sauphanor. 2001. Pleiotropy of insecticide resistance in the codling moth, *Cydia pomonella*. Entomologia Experimentalis et Applicata. 99(3): 381-386.

Boivin, T., J. Chadoeuf, J.C. Bouvier, D. Beslay and B. Sauphanor. 2005. Modelling the interactions between phenology and insecticide resistance genes in the codling moth *Cydia pomonella*. Pest Management Science. 61(1):53-67.

Bouvier, J.C., T. Boivin, D. Beslay and B. Sauphanor. 2002. Age-dependent response to insecticides and enzymatic variation in susceptible and resistant codling moth larvae. Archives of Insect Biochemistry and Physiology. 51(2): 55-56.

Bouvier, J.C., R. Buès, T. Boivin, L. Boudinhon, D. Beslay and B. Sauphanor. 2001. Deltamethrin resistance in the codling moth (Lepidoptera: Tortricidae): inheritance and number of genes involved. Heredity. 87(4): 456-462.

Brunner, J., S. Welter, C. Calkins, R. Hilton, E. Beers, J. Dunley, T. Unruh, A. Knight, R. Van Steenwyk and P. Van Buskirk. 2002. Mating disruption of codling moth: a perspective from the Western United States. IOBC wprs Bulletin 25.

Dunley, J.E. and S.C. Welter. 2000. Correlated Insecticide Cross-Resistance in Azinphosmethyl Resistant Codling Moth (Lepidoptera: Torticidae). Journal of Economic Entomology. 93(3): 955-962.

Howell, J.F. and L.G. Neven. 2000. Physiological development time and zero development temperature of the codling moth (Lepidoptera: Tortricidae). Environmental Entomology. 29(4): 766-772.

Knight, A.L., J.E. Dunley and R.K. Jansson. 2001. Baseline monitoring of codling moth (Lepidoptera: Tortricidae) larval response to benzoylhydrazine insecticides. Journal of Economic Entomology 94(1): 264-270.

McKenzie, J.A. and P. Batterham. 1994. The genetic, molecular and phenotypic consequences of selection for insecticide resistance. Trends in Ecology and Evolution. 9(5): 166-169.

Sauphanor, B., M. Benoit, J.C. Bouvier, G. Perron, S. Malezieux and J.C. Fremond. 1995. Cross-resistance between benzoylureas and benzoylhydrazines in the codling moth, *Cydia pomonella* L. Pesticide Science. 45(4): 369-375.

Sauphanor, B., V. Brosse, C. Monier and J.C. Bouvier. 1998. Differential ovicidal and larvicidal resistance to benzoylureas in the codling moth, *Cydia pomonella*. Entomologia Experimentalis et Applicata. (88): 247-253.

Sauphanor, B., A. Cuany, J.C. Bouvier, V. Brosse, M. Amichot and J.B. Bergé. 1997. Mechanism of resistance to deltamethrin in *Cydia pomonella* (L.) (Lepidoptera: Torticidae). Archives of Pesticide Biochemistry & Physiology. 58(2): 109-117.

Walker, K.R. and S.C. Welter. 2001. Potential for outbreaks of leafrollers (Lepidoptera: Torticidae) in California apple orchards using mating disruption for codling moth suppression. Journal of Economic Entomology 94(2): 373-380.

Welter, S.C. and F. Cave. 2004. Insecticide resistance in codling moth: resistance to new selective chemistries and potential changes in developmental rates. Pp. 70-81 *In* California Pear Research Report 2004. California Pear Advisory Board, Pear Pest Management Research Fund.