PHENOLOGY MODEL FOR WALNUT HUSK FLY

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ABSTRACT

Walnut husk fly (*Rhagoletis completa*, WHF) is an invasive pest that originates from the eastern United States. It was first found in California in the 1920's and has subsequently spread throughout the western region. WHF is a mid to late-season pest with one generation each year. As WHF has no effective natural enemies in California management is based on insecticide treatments. The timing of insecticide treatments for other tephritid fruit fly pests in tree crops in the western region is based on the use of degree-day phenology models. Phenology models are currently used in the management of the closely related apple maggot (*Rhagoletis pomonella*) and western cherry fruit fly (*R. indifferens*).

Further investigation of the relationship between the rate of development (time to emergence) of overwintered WHF puparia in relation to temperature showed that there was no effect of soil moisture (dry versus 9% moisture content) or walnut cultivar (a comparison of puparia from Black Walnut, Chandler and Chico). Thus for WHF populations in California that have been chilled for 120 days have a lower temperature threshold for development of 2.36 °C and a thermal requirement for adult emergence of 2273 degree days. The thermal requirement is influenced by winter chilling, with greater levels of chilling reducing the thermal requirement to a minimum of 968 degree days. Through collections of puparia from different walnut cultivars we found that females emerge earlier from puparia of smaller size (fresh weight), suggesting that there may be a nutritional effect associated with the nuts of different cultivars. We also found some evidence of a latitudinal effect on puparial weight for puparia collected from Black Walnut. The preoviposition period for female WHF ranged from 250-400 degree days (9-23 days) and was found to declined with nut maturity.

Using an extended data set of historical WHF trap catch records from 128 orchard years with broader regional coverage over a period of 17 years, we were able to estimate that on average the accumulated degree days from March 1 above a lower threshold of 5 °C for 50% cumulative trap catch was 2208 (± 260 SD). This value is very similar to the 2273 degree days estimated from our laboratory observations. We used 91 of the orchard year records to develop phenology models for 5%, 50% and peak trap catch, based on the number of chill hours during winter and the level of precipitation in January and February from nearby CIMIS stations. Trap catch records from the remaining 37 orchard years were used to test the phenology models. For all three of these measures of the seasonal flight activity of WHF, the phenology models provided a good match between predicted and observed degree days. The average difference between predicted and observed timing for the test orchard years was less than 46 degree days which corresponds to less than 2 days.

OBJECTIVES

1) Develop a preliminary phenology model for WHF based on 16 years of trap catch and temperature data from Red Bluff, CA and thermal constants from OR
2) Extend estimation of threshold temperatures and thermal requirements for WHF populations in California to include varying chilling times and moisture conditions
3) Continue to investigate effects of irrigation and cultivar on accumulated degree-day requirements for WHF to improve the phenology model
4) Validate the improved model using additional trap catch data from walnut orchard locations in the Central Valley

This year we focused on Objectives 2-4, having addressed Objective 1 last year.

SIGNIFICANT FINDINGS

- Soil moisture does not influence the temperature accumulation of WHF puparia after overwintering
- The thermal requirement for WHF emergence from overwintered puparia depends on the extent of winter chilling
- Female WHF from smaller puparia emerge earlier than those from larger puparia
- Cultivar and latitude may have an important influence on puparial weight
- Phenology models have been developed to predict the degree day timing for 5%, 50% and peak trap catch based on winter chill hours, precipitation in January and February, and random effects associated with location

PROCEDURES

Objective 2: Extend estimation of threshold temperatures and thermal requirements for WHF populations in California to include varying chilling times and moisture conditions

All WHF puparia collected from several walnut orchards in California during the summer of 2014 were overwintered and chilled at 6 °C in sandwich boxes with a 5cm layer of a 3:2 peat moss:sand mix at the Insectary and Quarantine facility at the University of California, Berkeley. Puparia used for laboratory experiments in 2015 were collected from Black Walnut and Chandler near Davis, CA on August 24-25, 2014.

In 2014 we evaluated the relationship between development rate (time to emergence) and temperature for overwintered puparia that had been chilled for 120 days to estimate the lower threshold temperature for development and the thermal requirement for adult emergence. To determine whether soil moisture could influence this relationship we repeated these laboratory observations from 2014, but 120 day chilled puparia were placed into cups with a 5cm layer of a 3:2 peat moss:sand mix that was kept at 9% moisture content. The 9% moisture content is based on research indicating that field capacity for sandy soils is 10% and thus 9% represents a moisture level that is just below field capacity. Ten replicate cups of 10 overwintered puparia collected from either Black Walnut or Chandler in 2014 were placed at a series of five constant temperatures from 12 – 24 °C, representing the linear part of the relationship between development rate and temperature, and checked daily for adult emergence. Pupae were monitored until no new flies had emerged for at least three weeks and median emergence time was recorded for each replicate petri dish.

Although 120 days of chilling is often used as a standard time period for overwintering insects, the number of chilling hours between 0 – 7.2 °C in California suggests that 60 days of chilling would be more representative of natural conditions. Therefore, we repeated our observations of the relationship between development rate (time to emergence) and temperature for WHF puparia that had been chilled for 60 days rather than 120 days. After chilling, puparia were removed from cold storage and were dipped in a 5% bleach solution to kill any mites and fungus present on the puparial casing. Ten individuals were placed in each of 10 replicate petri-dishes at a series of five constant temperatures from 12 – 24 °C. As in the previous experiment, pupae were monitored until no new flies had emerged for at least three weeks and median emergence time was recorded for each replicate petri dish.

Little is known about how chill accumulation influences overwintering diapause in insects, and whether post diapause heat accumulation is initiated only after diapause has terminated. Recent research from tree crops suggests that tree buds have both a maximum requirement for heat accumulation when they experience no chilling, and a minimum requirement for heat accumulation when they experience excess chilling. The actual timing of bud burst lies between the maximum and minimum requirements and appears to be determined by a complex linkage between chill and heat accumulation. To determine the minimum requirement for heat accumulation for overwintered WHF puparia, we used a set of puparia that had been collected from Black Walnut and Chandler in the summer of 2013. These puparia had been chilled for over a year before being placed in replicate petri dishes at 24 °C to await emergence. As in the previous experiments, median emergence time was recorded for each replicate set of WHF puparia.

WHF adults vary considerably in size, and as is typical of insects, larger female size tends to be associated with greater egg laying capacity. To determine whether size influences the timing of emergence from overwintered puparia we evaluated the relationship between puparial weight and emergence time. After 135 days of chilling 200 Chico puparia were individually weighed (in mg) and tracked over their development. Individually-labeled gel caps with one puparium each were kept at 24 °C and monitored for emergence time. Emerging flies were then sexed and degree days at emergence was calculated.

Although the timing of adult emergence represents the start of WHF activity for the season, there is a notable delay between emergence and oviposition during which females feed to provide the nutrients needed to mature their eggs. The duration of this preoviposition period is also temperature dependent and was evaluated in terms of degree days at 24 °C. Each date if 5 flies or more emerged they were placed in a 47 cm^3 mesh cage with a fresh green walnut. Flies used in this experiment emerged from a wide variety of experiments conducted in 2014 on temperature, moisture and chill time. Cultivars included were Black Walnut, Chandler, Chico, Hartley, and Vina. Flies were provided with a diet of fructose:yeast hydrolysate:water (7:4:10) as well as water. Diet was provided by dipping a cotton dental wick in the diet solution and hanging it from the top of the cage using a paperclip. Water was available via a dental wick partially submerged in a small capped solo cup filled with water and placed on the bottom of the cage. Diet was replaced every 2-3 days and the water reservoir on the bottom of the cage was refilled as necessary. Walnuts were inspected daily for stings (oviposition sites) and first oviposition was verified by using a scalpel to check for the presence of eggs.

Objective 3: Continue to investigate effects of irrigation and cultivar on accumulated degree-day requirements for WHF to improve the phenology model

Data from experiments conducted last year suggested that there may be a difference in the thermal requirement for adult emergence between WHF puparia collected from different walnut cultivars. To determine whether WHF larvae that feed in the husks of nuts from different cultivars develop to produce puparia of different weights we collected from Black Walnut, Hartley, Payne, Tehama, and Vina in August 2015. Puparia were collected from 10 orchards at 5 different latitudes through the Central Valley (Chico, Winters, Brentwood, Stockton and Hollister). All pupae are currently being chilled at 6 $^{\circ}$C for 90 days and will be moved to a 24 $^{\circ}$C incubator after chilling. A subset of 100 puparia from each of the cultivars were individually weighed before chilling, and the data for Black Walnut collected from each of four locations are presented here.

An irrigation trial has also been set up with puparia collected in September 2015. A set of 2,000 puparia, collected from Tehama near Chico, were separated into groups of 50 and used in a field experiment with 20 replicates of each of two irrigation treatments. The irrigation treatments consist of a control exposed to natural winter and spring rainfall only and a flood irrigation treatment exposed to natural rainfall and supplemented with 2-3 irrigation events (5 cm water) spaced at 3 week intervals starting in early May 2016. Puparia for this experiment were buried 2cm deep in the soil of 0.11m^2 plots at the Oxford Tract at UC Berkeley on November 1 to coincide with the start of the winter chill period. Emergence of WHF adults will be monitored every two days from May through August 2016 to record both timing and survivorship.

Objective 4: Validate the improved model using additional trap catch data from walnut orchard locations in the Central Valley

For objective 1, last year we analyzed 14 years of trap catch data from a single orchard in Red Bluff to determine the degree day requirement for adult emergence. This year we added an additional 115 orchard years to the data set, based on WHF trap catch data from Tehama, Shasta, Butte, Sutter and San Joaquin counties between 1999 and 2014. Three criteria were used to ensure that the trap catch data for a particular location and year were of sufficient quality to be included in our data set. Firstly, at least 50 flies must have been caught over the duration of the season. Secondly, the trap catch records must have one date either at the start or end of the season when no flies were caught to fix either the beginning or end of the flight period. Thirdly, if the other end of the flight period did not have a zero trap catch date, that date could not account for more than 2% of total trap catch over the season. After these criteria for inclusion had been applied our data set consisted of 128 orchard-years representing a 15 year period and latitudes from Shasta to San Joaquin counties. Each orchard-year in the data set was matched to the prevailing climate data from the nearest CIMIS (California Irrigation Management Information System) station.

Using historical data from the nearest CIMIS station, temperature accumulation was calculated beginning March 1 of each year, a date that has been used in phenology models for apple maggot, western cheery fruit fly and blueberry maggot. We also considered temperature accumulation from January 1 and May 1, but March 1 provided a better match to our laboratory estimates of the thermal requirement for adult WHF emergence. Accumulated degree days were calculated using the University of California Integrated Pest Management Degree Day calculator based on the single sine method with upper and lower temperature thresholds of 30 °C and 5 °C, respectively. These thresholds had been estimated in the 1990s from research conducted on WHF in Oregon. The relationship between cumulative percent trap catch and accumulated degree days from March 1 was fitted to a cumulative Weibull function $f(\chi) = 1 - \exp(-\chi/\lambda)^{\kappa}$. This function describes an S-shaped curve for which $f(\chi)$ is cumulative percent trap catch at time χ (in degree days), while λ is a scale factor that shifts the curve along the horizontal axis, and κ is a shape factor that varies from 1 to ∞ and influences the spread of the curve.

To develop a predictive phenology model for WHF in California we used linear mixed effects models developed in R with the lme4 package. Linear mixed effects models are ideal for analyzing the phenology of trap catch for WHF because they are effective in dealing with unbalanced data and random effects. We related four different aspects of the timing of cumulative trap catch through the season to environmental factors in the preceding year; 5%, 50% and peak trap catch and the interval between 5 and 95% trap catch. We used the timing for 5% cumulative trap catch rather than the first fly caught to predict the start of the WHF flight period. As for codling moth trap catch, aberrant individuals of WHF can be caught much earlier in the season than the rest of the flies and are not considered to be representative of the start of the main flight period. We used the timing for 50% cumulative trap catch to represent the mid-point of the flight period, and the timing of peak trap catch to represent the period of greatest flight activity. We also used the period from 5-95% cumulative trap catch to represent the spread of the flight period, but were unsuccessful in relating this to any of the environmental factors considered.

The predictive environmental factors that we considered for the phenology models included both measured climatic variables as fixed factors and unknown variables specific to individual orchards as random factors. Previous research has shown an effect of chill hours during winter (cumulative hours between 0 – 7.2 °C from November until the end of March) on the thermal requirement for adult emergence of the closely related apple maggot and western cherry fruit fly. Additionally, rainfall and soil moisture have been shown to influence the thermal requirement for emergence of apple maggot, but effects on western cherry fruit fly appear variable. Based on this earlier research, we included chill hours and January-February precipitation (with an interaction term) as fixed effects in the phenology models for WHF. We also included a random intercept for year nested within CIMIS station to account for the lack of independence in the climatic data. This is due to the structure of the trap catch data set; orchards that are closer in proximity share the same CIMIS station and thus share the same climatic data for a particular year. Visual inspection of residual plots for the fitted models did not reveal any obvious deviations from homoscedasticity or normality, and P-values were estimated based on Satterthwaite's approximations.

It is best practice to develop a phenology model using only approximately two thirds of the full data set, and then to evaluate the model against the remaining third of the data set. We separated the full data set to maximize the variation in climatic data represented in the subset used for the development of the phenology models. This included all year/CIMIS station combinations for which there were less than three associated orchards and a randomly selected two thirds of the year/CIMIS station combinations for which there were three or more associated orchards (n = 91). The phenology models were then used to predict the timing of 5%, 50% and peak trap catch for the remainder of the data set (n = 37) that was not included in the building of the models. The predicted degree day timing was then compared to the observed degree day timing and quantified using the mean difference between predicted and observed values.

RESULTS

Objective 2: Extend estimation of threshold temperatures and thermal requirements for WHF populations in California to include varying chilling times and moisture conditions

We examined the effect of soil moisture on the relationship between rate of development (time to emergence) and temperature for overwintered WHF puparia chilled for 120 days. We compared data from puparia collected from Chico in 2013 and kept in dry petri dishes after chilling, with puparia collected from Black Walnut and Chandler in 2014 and kept in peat moss:sand medium maintained at a 9% moisture content (Fig. 1). There was no evidence of any difference in development rates in relation to temperature for either moisture content or cultivar. A single pooled model was fitted to these three data sets and this model estimates the lower temperature threshold for development to be 2.36 °C and the thermal requirement for adult emergence to be 2273 degree days.

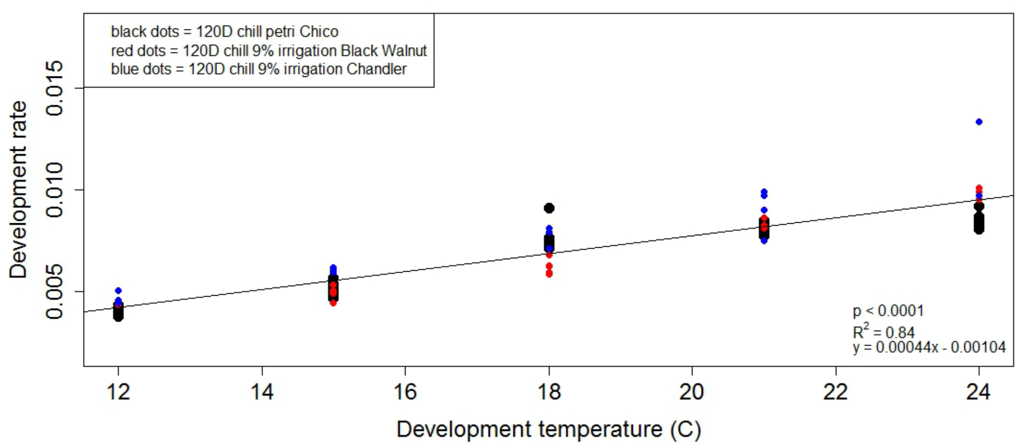

Fig 1. The relationship between developmental rate (1/development time) and temperature for overwintered WHF puparia These data are for puparia chilled for 120 days and either collected from Black Walnut and Chandler in 2014 and kept at 9% moisture, or from Chico in 2013 and kept in dry petri dishes.

For a shorter chilling time of 60 days the relationships between development time and temperature for puparia collected from Black Walnut and Chandler in 2014 and kept in dry petri dishes were very similar and fitted to a single pooled model. This model estimates the lower developmental threshold to be 5.47 °C and the thermal requirement for adult emergence to be

2041 degree days (Fig. 2). The unexpectedly low estimate for the thermal requirement results from the greater slope for this relationship compared to the equivalent one for 120 days chilling (Fig. 1). The slope is very sensitive to outliers at either the lowest or highest temperatures tested and may have been unduly influenced by the earlier than expected emergence from some of the replicate petri dishes at 24 °C.

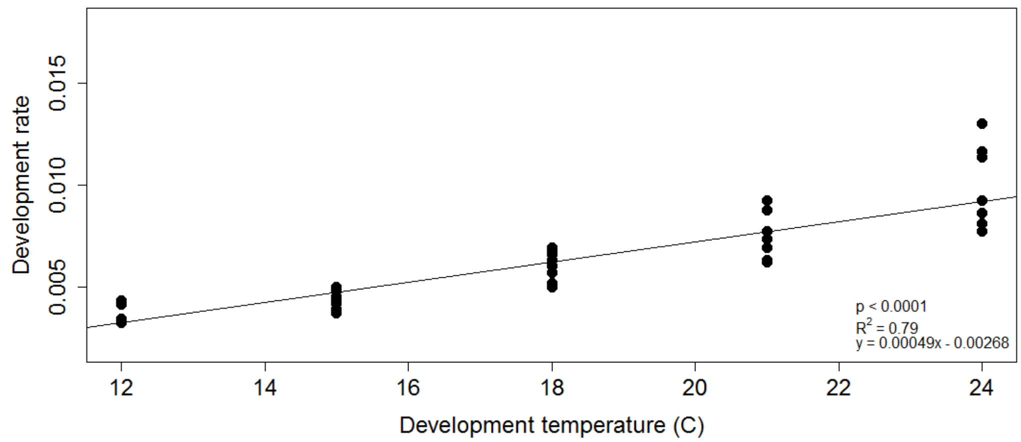

Fig 2. The relationship between developmental rate (1/development time) and temperature for overwintered WHF puparia. These data are for puparia chilled for 60 days and collected from Black Walnut and Chandler in 2014 and kept in dry petri dishes.

Puparia collected in 2013 from Black Walnut and Chandler were chilled for over a year to evaluate the minimum thermal requirement for emergence after excess chilling. There was no difference between cultivars in the degree day requirement for median emergence (Fig. 3), which averaged 968 degree days (SD = 68), a value much closer to the estimated thermal requirement for WHF populations in Oregon.

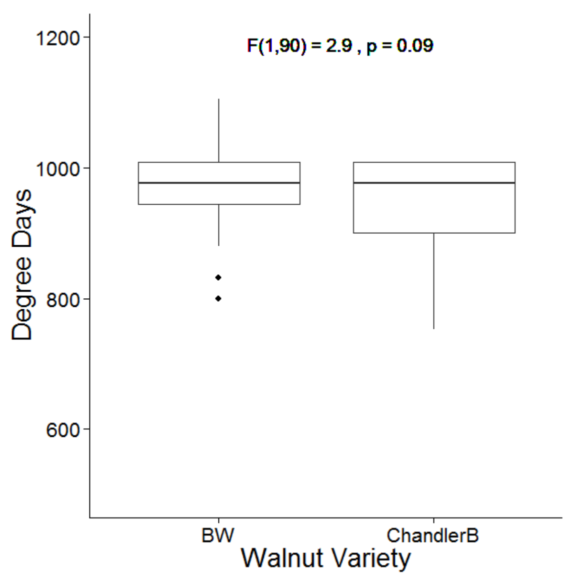

Fig. 3. Minimum requirement for heat accumulation after more than one year of chilling for WHF puparia collected from Black Walnut and Chandler in 2013 and kept in dry petri dishes.

When emergence times were monitored in the laboratory for individual WHF in relation to puparial weight there appeared to be a strong positive correlation for female flies (Fig. 4). Females emerged earlier from lighter than from heavier puparia. This variation in pupal weight can potentially be explained by differences in the nutritional value of different cultivars for WHF larvae or by competition between larvae sharing a single nut. Ongoing research is exploring the possibility that puparial weights differ between walnut cultivars and that this may be one of the

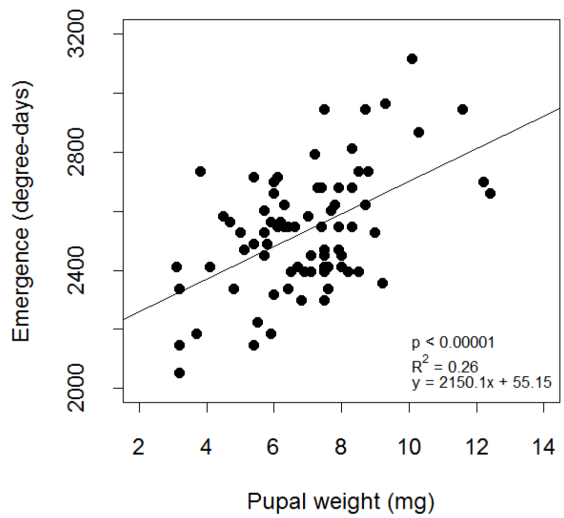

Fig. 4. Accumulated degree days at emergence for female WHF in relation to puparial weight.

factors causing variation in the timing of trap catch among orchards for the same latitude and year (see Objective 4 below).

The preoviposition time (in degree days) was measured for females in relation to nut maturity over the period from late June through early August. Although there was considerable variation in the data obtained, there was a significant effect of nut maturity on preoviposition time, indicating that emerging females reached oviposition status more quickly when exposed to nuts of greater maturity (Fig. 5). This suggests that if flies emerge early in an orchard of a late-maturing cultivar they are likely to experience a longer preoviposition period, greater mortality before egg laying, and a reduced egg laying capacity. Preoviposition times ranged from 200-450 degree days, and assuming that 20-23 degree days are accumulated per day in June/July, this corresponds to a period of 9-23 days.

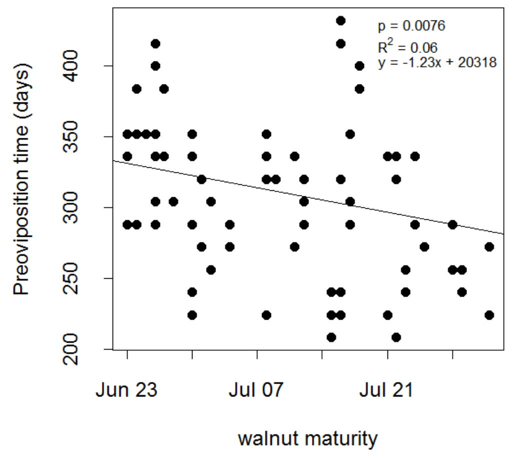

Fig. 5. Relationship between preoviposition time for WHF females and nut maturity as represented by date collected from the field.

Objective 3: Continue to investigate effects of irrigation and cultivar on accumulated degree-day requirements for WHF to improve the phenology model

Based on an apparent difference in the thermal requirements for emergence observed for WHF collected from different cultivars last year, and the correlation between puparial weight and female emergence (Fig. 4), we further explored patterns of puparial weight among cultivars controlling for collection date and latitude. Our preliminary data for Black Walnut do show some differences in puparial weight with latitude (Fig. 6), and next summer we will be able to monitor their emergence having been overwintered under the same conditions. We are also evaluating puparia collected from different cultivars at the same latitude.

We have set up a replicated field experiment in October 2015 to monitor the effects of simulated irrigation events on the timing of emergence of WHF overwintered in a natural soil environment. The irrigation events will be applied early next summer and emergence times monitored in field cages.

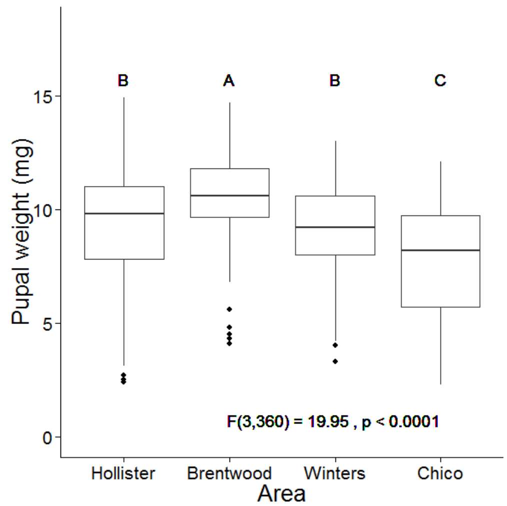

Fig 6. Puparial weight of WHF collected from Black Walnut for four orchard locations arranged southernmost to northernmost.

Objective 4: Validate the improved model using additional trap catch data from walnut orchard locations in the Central Valley

Using trap catch data from all 128 orchard-years included in our data set and a lower temperature threshold for development of 5 °C we determined that the accumulated degree days from March 1 for 5% cumulative trap catch averaged 1741 DD(°C) with a standard deviation of 221 DD, and for 50% trap catch averaged 2208 DD(°C) with a standard deviation of 260 DD (Fig. 7). Laboratory experiments on the influence of temperature on median (50%) emergence after 120 days of chilling suggested a lower threshold for development of 2.36 °C, and a thermal requirement of 2273 DD(°C), matching the trap catch estimate from the historical field data.

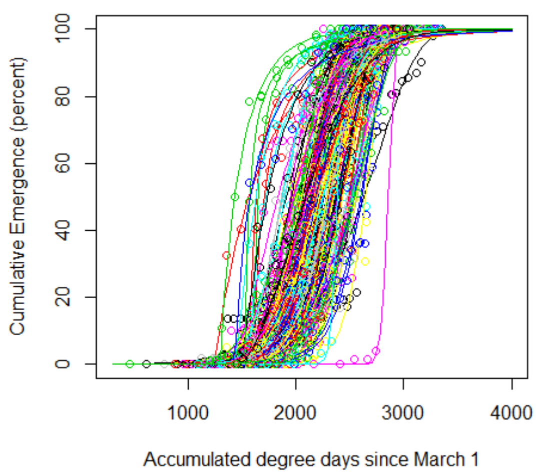

Fig. 7. Fitted cumulative distribution functions to describe the cumulative WHF trap catch for each of the orchard-years included in our expanded data set (n=128).

In developing phenology models for 5%, 50% and peak WHF trap catch we started by exploring the effects of individual climatic variables. For example, there is a significant relationship between 50% cumulative trap catch and chill hours (Fig. 8), however, as very little of the variance in trap catch was explained by chill hours alone the next step was to developed a more complex model that included other environmental factors. As our previous analysis of 14 years of trap catch data from a single orchard in Red Bluff indicated a relationship between spring rainfall and trap catch (Walnut Research Report 2014), we evaluated various configurations of spring rainfall in conjunction with chill time to select the most accurate model.

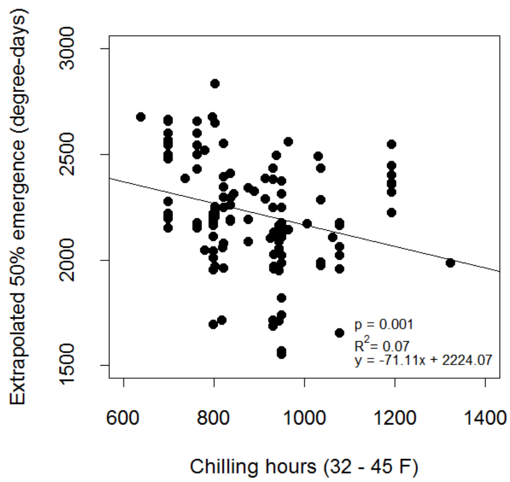

Fig. 8. Linear relationship between 50% cumulative trap catch and chilling hours for the 91 orchard-years used for phenology model development.

Our current WHF phenology models are based on accumulated degree days at 5%, 50% or peak trap catch as the response variable, precipitation in January and February, chilling hours (0 – 7.2 °C between Nov 1-Feb 28) and an interaction term as the independent fixed effect variables, and a random slope with year nested within CIMIS station to account for the lack of independence in the climatic data. It was necessary to rescale the precipitation and chilling hours data to be centered at zero and have a variance of one. In all three models both chilling hours and the interaction term between in trap catch chilling hours and precipitation were significant with $P < 0.05$. The amount of the variance in degree days explained by the fixed effects of the 5% trap catch model was 13% and was increased to 66% when the random effect was taken into consideration. For the 50% trap catch model 19% of the variance was explained by the fixed effects alone and 35% was explained when the random effects were included. Finally, for the peak trap catch model 18% of the variance was explained by the fixed effect alone and 22% when the random effects were included.

After the three WHF phenology models had been evaluated for goodness of fit they were subsequently tested against the 37 orchard years that were not included in the development of the models. For this test, the phenology models were used to predict values for accumulated degree days at 5%, 50% and peak trap catch for each of the 37 orchard years, based on their associated climatic data and random effects. A comparison of observed and predicted degree days show a good match for all three measures of phenology (Fig. 9a,b,c). The mean differences between

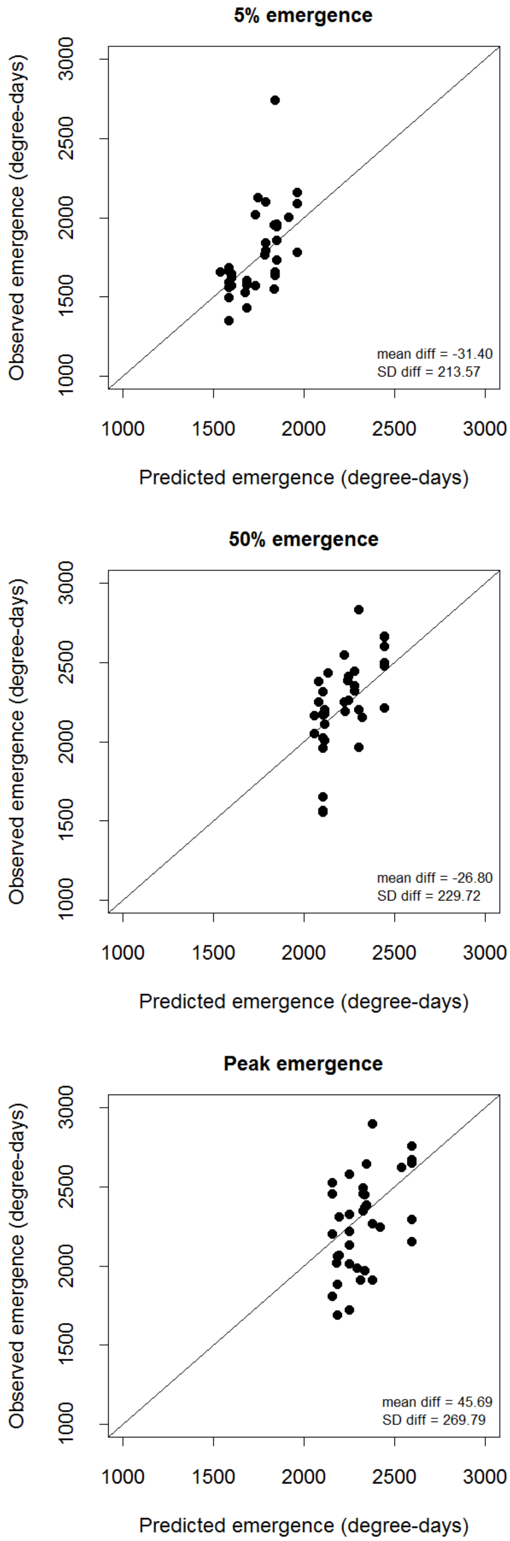

Fig. 9. Evaluation of the phenology models for WHF trap catch, showing observed and predicted degree days for the timing of (a) 5%, (b) 50%, and (c) peak trap catch.

observed and predicted degree days were negative for both 5% and 50% cumulative trap catch indicating that the predicted values are slightly overestimated by the phenology models, while that for peak emergence was positive suggesting slight underestimation. In all three cases the mean differences are less than 46 degree days, and assuming that 20-25 degree days above a threshold of 5 °C are accumulated on an average day in June/July, this amounts to no more than 2 days mismatch in the predictions of the phenology models.

DISCUSSION

From our laboratory observations on the effect of temperature on the rate of development (timing of emergence) of overwintered WHF puparia we have been able to determine that soil moisture does not influence the process of temperature accumulation in spring and early summer. This is an important finding as WHF puparia survive much better under drier conditions in the laboratory and we typically keep the puparia in dry petri dishes as a result. We currently know nothing about how precipitation or irrigation events affect the survivorship of WHF puparia under field conditions and this should be investigated in the future. We currently have a field experiment set up in the field that will allow us to monitor survivorship under conditions of natural precipitation versus natural precipitation plus 2-3 flood irrigation events in early summer, and this will be monitored through the 2016 field season.

Our laboratory observations using lower levels of chilling for the overwintering of WHF puparia to better match the natural conditions during winter in California provided some anomalous observations during 2015 and an additional experiment on the effects of chilling time has been set up in November this year to repeat these observations in 2016. By weighing individual puparia we found evidence this year of a relationship between the timing of adult female emergence and the size of the puparia, with larger females emerging later than smaller females. Coupled with a possible effect of cultivar on puparial weight this could help to explain some of the observed variation in the timing of trap catch records from neighboring orchards in the same walnut growing regions. This will be explored in greater detail in 2016. We also found evidence this year that the preoviposition period of WHF females, the period between emergence and the start of egg laying, was not only very variable (from 9-23 days), but also appeared to be influenced by the maturity of the nuts presented to them in laboratory cages. This effect of nut maturity suggests that the preoviposition period may vary between walnut cultivars and will again be explored in greater detail in 2016.

Through access to an extended data set of historical trap catch records for WHF that cover a broader range of walnut growing regions we have been able to develop a more effective set of phenology models for flight activity through the season. These models are based on degree day accumulation from March 1 above a lower temperature threshold of 5 °C and include winter chill hours, precipitation in January and February and random effects of location as explanatory variables. The phenology models were tested using a part of the historical trap catch data set that were not used for model development, and the predicted degree days for 5%, 50% and peak trap catch were all well matched to the observed degree days in these orchard years. Although the average difference between predictions and observations was only approximately 2 days, the models could be further improved through addition of information on exact latitude, cultivar and orchard age. We will continue to explore this in 2016.

CONCLUSION

Adult emergence of WHF is primarily driven by degree day temperature accumulation and the effects of winter chilling and precipitation, and consequently our current phenology models provide a good preliminary estimate of the timing of 5%, 50% and peak trap catch through the season. Little is known about the preoviposition period for WHF and initial observations suggest that it may be longer earlier in the season and influenced by nut maturity. Further details of the influence of cultivar and precipitation on both the timing of adult emergence and the survivorship of puparia in the soil would be of value in the development of a more effective management program for WHF in the future.