

PHENOLOGY MODEL FOR WALNUT HUSK FLY

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ABSTRACT

Walnut husk fly (*Rhagoletis completa*, WHF) has become an increasingly important pest in walnuts in recent years, causing greater concern for growers and leading to more frequent insecticide spray applications for its successful management. The goal of this project is to develop more accurate methods for predicting the timing of WHF emergence to eliminate unnecessary sprays, reduce pesticide loading of the environment, and reduce the potential for resistance development. Spray timing for other tephritid fruit fly pests in tree crops in the western region is predicted by use of degree-day phenology models. While phenology models have been developed and are currently used for codling moth management in walnuts, there is currently no model to facilitate management of WHF.

Based on analysis of 12 years of WHF trap catch data from Red Bluff and using temperature thresholds from a study of WHF in Oregon (5 and 30°C), first emergence of adults from overwintering puparia occurred between 1150 and 1500 degree days centigrade (DD°C) after March 1. Though there was considerable year to year variation in the timing of adult emergence, it appeared that rainfall could explain some of this variation. When male and female emergence was combined there was a significant positive relationship between the degree days for first emergence and annual rainfall. However, for females alone, there was greater variability in the degree day accumulation needed for first emergence in low rainfall years converging on the mean in higher rainfall years. In addition, the variation in emergence timing for females could be explained by the extent to which January to June rainfall was concentrated into the months of March and April.

Laboratory experiments were conducted to further delineate the sources of variability in the timing of adult WHF emergence. In contrast to WHF from Oregon, overwintered WHF puparia from California had a slightly lower developmental threshold (3.7 versus 5°C), and a much greater requirement for accumulated degree days emergence (2040 versus 976 DD°C). Irrigation or rain events that occurred 6 and 8 weeks after termination of puparial diapause delayed the emergence of adult WHF in the laboratory. In contrast, increasing chill time for overwintering puparia reduced the subsequent degree day accumulation required for adult emergence. Finally, walnut cultivar also appeared to significantly influence the timing of WHF emergence.

OBJECTIVES

The overall goal of this project is to provide growers and PCAs with a more accurate way to predict the timing of adult WHF emergence. Accurate timing of WHF emergence is critical for effective spray treatments and would also help to eliminate unnecessary sprays, reduce pesticide loading of the environment, and reduce the potential for resistance development. Specific objectives are to:

- 1) Develop a preliminary phenology model for WHF based on 17 years of trap catch and temperature data from Red Bluff, CA and thermal constants from OR
- 2) Estimate threshold temperatures and thermal requirements for WHF populations in California to improve the preliminary phenology model
- 3) Investigate effects of chill time, irrigation and cultivar on accumulated degree-day requirements for WHF to improve the phenology model
- 4) Validate the improved model using new trap catch data from walnut orchard locations in the Central Valley

During this first year of the project, we focused on the first three of these objectives.

SIGNIFICANT FINDINGS

- The timing of emergence of adult walnut husk fly is primarily driven by degree day accumulation of puparia once overwintering diapause is completed, and is earlier for males than for females.
- For both sexes combined the degree days to first adult emergence is positively related to annual rainfall, but that of females alone shows greater variability in low rainfall year, with earlier first emergence in low rainfall years when January-June rainfall is concentrated in March and April.
- Relative to WHF in Oregon, California populations have a slightly lower developmental threshold temperature for puparial development of 3.7°C and require twice as much accumulated degree days for adult emergence at 2040 DD°C.
- Laboratory experiments suggested that rain or irrigation events during post diapause puparial development of WHF can delay adult emergence, that increased chill time for WHF puparia can accelerate adult emergence, and that walnut cultivar can influence the timing of adult WHF emergence.

PROCEDURES

Obj. 1. Develop a preliminary phenology model for WHF based on 17 years of trap catch and temperature data from Red Bluff, CA and thermal constants from OR

Adult WHF trap catch was recorded for 17 years (from 1998 to 2014) at a Red Bluff walnut orchard. Twelve years were selected for analysis in which more than 25 females were caught over the season. Degree-day (DD) accumulations were estimated using the single sine method with a vertical threshold cutoff with lower and upper temperature thresholds of 5 and 30°C as estimated for WHF populations in Oregon. Temperature data for the orchard in Red Bluff was based on the CIMIS (California Irrigation Management Information System) weather station in Gerber.

Temperature accumulation between the lower and upper threshold was estimated for a range of different initial start dates, and March 1 was selected as the most appropriate start date, based on the consistency of the DD accumulation for first and 50% trap catch between years for the Red Bluff data and the fact that this date has also been used successfully for phenology models developed for apple maggot and western cherry fruit fly. Separate phenology models were developed for total trap catch (both sexes combined) and for female trap catch only. The models were based on the relationship between cumulative trap catch and DD accumulation, and were fitted to a cumulative Weibull function $f(x) = 1 - \exp(-x/\lambda)^\kappa$. The cumulative Weibull function describes an S-shaped curve for which $f(x)$ is cumulative percent trap catch at time x (in DD), 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.

Obj. 2. Estimate threshold temperatures and thermal requirements for WHF populations in California to improve the preliminary phenology model

Since the thermal requirements for completion of puparial development were likely to be notably larger for California WHF due to decreased chill time compared to Oregon, we collected larvae from an orchard in northern California in the summer of 2013. The puparia were overwintered and chilled at 6°C in sandwich boxes with a 5cm layer of a 3:2 peat moss:sand mix in a cold room at the Insectary and Quarantine facility at the University of California, Berkeley. In 2014 we removed puparia from cold storage after 120 days of chilling and dipped them in a 5% bleach solution to kill mites and any fungi present on the puparial casing. Ten individuals were placed in each of 10 replicate petri-dishes at a series of seven constant temperatures from 12 – 30°C. The timing of median emergence from each petri-dish at each temperature was used to examine the relationship between the post-diapause development rate of overwintered WHF puparia and temperature. Puparia were monitored until no new flies had emerged for at least three weeks. A linear regression was fitted to the data over the lower range of temperatures in order to estimate the lower threshold temperature for development and the thermal requirement for adult emergence.

Obj. 3. Investigate effects of chill time, irrigation and cultivar on accumulated degree-day requirements for WHF to improve the phenology model

The effect of chill time has been shown to influence the DD accumulation necessary for emergence of many overwintering insects. Five treatments, with 50 WHF puparia each were exposed to 90, 105, 120, 135 and 150 days of chilling at 6°C before being moved to an incubator at 24°C to monitor the timing of first and median adult emergence. Similarly, to determine the effect of timing of simulated rainfall events on the emergence of WHF adults puparia were removed from the 6°C overwintering room after 105 days of chilling. Ten replicate sets of WHF puparia were placed in a 3:2 peat moss:sand mix in plastic cups, moved to an incubator at 24°C, and assigned to control (no irrigation, constant 9% moisture), early irrigation, mid-irrigation and late irrigation treatments. An irrigation treatment consisted of adding 100 ml of water, sufficient water to temporarily saturate the peat moss:sand medium, either 2 and 4 weeks (early), 4 and 6 weeks (mid) or 6 and 8 weeks after termination of diapause. For each treatment first and median adult emergence were monitored.

To evaluate the effect of walnut cultivar on the timing of adult WHF emergence, larvae were collected from orchards in northern California between August and September 2013. The orchards from which the infested walnuts were collected were located from Hollister to Red Bluff. Infested nuts were collected from six walnut cultivars (or species) including Black Walnut, Chandler, Chico, Franquette, Hartley and Vina. Two separate sets of infested Chandlers were collected from the same orchard almost a month apart. After pupation in sand and chilling at 6°C for 105 days, ten puparia from each cultivar were placed in a petri dish and ten replicate dishes per cultivar were moved to an incubator at 24°C to monitor the timing of first and median adult emergence.

RESULTS

Obj. 1. Develop a preliminary phenology model for WHF based on 17 years of trap catch and temperature data from Red Bluff, CA and thermal constants from OR

Based on the 12 years of trap catch data from Red Bluff our preliminary phenology model showed that the trap catch of WHF adults is primarily driven by DD accumulation, requiring over 2000 DD°C for 50% cumulative trap catch (Fig. 1). The fitted cumulative distribution functions indicate that there is about 500 DD°C of variability in the timing of 50% cumulative trap catch between years. As male flies emerge before females, there was also a slightly lower DD accumulation for 50% cumulative trap catch of male and female WHF combined (Fig. 1A) versus females alone (Fig. 1B).

To account for the variation between years in accumulated DD for trap catch of WHF, we evaluated other environmental variables in addition to temperature. We found no evidence that the extent of winter chill influenced the timing of trap catch, but that annual rainfall had a positive influence on the DD accumulation required for the timing of first WHF capture when male and female trap catch were combined (Fig. 2A). Thus the DD accumulation required for first WHF capture of the season increased from around 1200 DD°C in low annual rainfall years to around 1400 DD°C in high annual rainfall years. When female WHF alone were considered, however, a different pattern emerged (Fig. 2B). In general, there was no trend in the timing of first female capture in relation to annual rainfall, with a mean DD accumulation of nearly 1400 DD°C, but there was much greater variability (over 400 DD°C) in the timing of first female capture in years with lower annual rainfall. To investigate this further, we looked at the distribution of rainfall in the months from January to June (spring rain). While the extent of rainfall declined progressively through this period, the variability in rainfall between years during the months after completion of WHF puparial diapause (March 1) was found to be much greater in March/April than in May/June (Fig. 3). In fact the extent of mid spring rainfall in March and April varied from 0-50% of the total spring rainfall from January to June. The relative concentration of spring rainfall in March and April (mid spring rain) could account for the variation around the mean DD accumulation for first female capture between years at Red Bluff (Fig. 4B). Years with a greater concentration of spring rainfall in March/April lead to earlier first capture than years with a lower concentration of spring rainfall in March/April. However, for first WHF capture (either male or female), annual rainfall alone accounted for the variation in DD accumulation between years (Fig. 2A) and mid spring rain did not add to the predictive effect of rainfall on the timing of first trap capture (Fig. 4A).

Obj. 2. Estimate threshold temperatures and thermal requirements for WHF populations in California to improve the preliminary phenology model

There was a linear relationship between the development rate of post diapause puparia and temperature in our laboratory study with WHF puparia collected from California (Fig. 5). The linearity of this relationship was only apparent over the lower range of temperatures (12 – 21°C), as is typical for insect studies, but it allowed the estimation of both the lower temperature threshold for puparial development (3.7°C) and the thermal requirement for adult emergence (2040 DD°C). At temperatures above 21°C, the development rate of WHF puparia slowed down and reached a maximum, as is typical for other insect studies. It is also important to note, that at the time of this report, adult WHF were still emerging from the 12°C treatment, and thus the estimates of the thermal requirements for California WHF are based on current rather than complete data.

Obj. 3. Investigate effects of chill time, irrigation and cultivar on accumulated degree-day requirements for WHF to improve the phenology model

Our other laboratory experiments on the effects of irrigation, chill time and walnut cultivar on the timing of adult WHF emergence from post diapause puparia are based on the DD accumulation for median emergence time of both sexes combined. For the simulated irrigation experiment, emergence occurred at a lower DD accumulation (just over 1800 DD°C) than expected, but rates of emergence were notably lower than for the other laboratory experiments. In addition, when irrigation events were timed at 6 and 8 weeks after termination of diapause, emergence was significantly delayed by more than 200 DD°C (Fig. 6). We also found that increased chill time decreased the DD accumulation necessary for adult emergence from post diapause puparia (Fig. 7). While this relationship appeared linear, it could also represent a step function where DD accumulation was generally higher for chill times below a threshold of around 112 days and lower for chill times beyond this threshold.

Finally, our results showed that there was a significant effect of cultivar on the accumulated DD for adult emergence from post diapause WHF puparia (Fig. 8). Particularly interesting is that WHF emergence from both Vina and Hartley was earlier than from the other cultivars tested. However, it is important to approach these results with caution, as in addition to cultivar, the timing of larval collection from the field and latitudinal variation in the location of the source orchards could also play a role in influencing the timing of WHF emergence. For example, the two groups of WHF larvae from Chandler were collected from the same orchard, but one month apart, and showed significantly different timing of emergence (Fig. 8).

DISCUSSION

Based on the analysis of 12 years of trap-catch data from Red Bluff as well as experiments on adult emergence from puparia subjected to different laboratory treatments, there appear to be a number of factors that can influence the timing of WHF emergence. The most important factor is accumulated degree days post diapause. From our preliminary analysis, based on trap catch data over a number of years from a single orchard in Red Bluff, it seems likely that overwintering puparial diapause is completed by March 1, and that 1400 DD°C need to be accumulated for first

trap catch, with 50% trap catch occurring around 2200 DD°C. First trap catch can occur earlier in years with low annual rainfall, and if the two sexes are distinguished in trap catches, first female capture can be better predicted by the relative concentration of spring rainfall (January to June) during the months of March and April. The generality of these initial results needs to be treated with caution, as they are representative of a single location only, and further analysis will be needed using trap catch data from a broader range of locations throughout the walnut growing regions of the Central Valley. At least one PCA group has indicated that they would be willing to share WHF trapping data with us and with a broader geographic data set this would allow us to include other factors such as latitude, soil, irrigation schedules and cultivars into our validation of the phenology model.

Laboratory experiments at constant temperatures allowed us to estimate the lower temperature threshold for WHF puparial development as 3.7°C, which is very close to the 5°C threshold estimated previously for WHF puparia in Oregon. In contrast, the thermal requirement for adult emergence from puparia was estimated to be 2040 DD°C as compared to the estimate of 976 DD°C for Oregon, indicating the importance of latitude as an influence on the phenology of WHF emergence. Laboratory experiments also confirmed the importance of increased chill time in reducing the DD accumulation required for adult emergence from puparia. This is of importance as it suggests that our estimate of 2040 DD°C for the thermal requirement of California WHF puparia may be an underestimate, with respect to field conditions, as it was based on an overwintering chill time of 120 days. A recently obtained dataset of hourly soil temperatures in Red Bluff for the winter of 2013-14, though not yet analyzed in detail, suggest that winter chill may actually have been as little as 60 days. A shorter chill time of 60 days would result in a greater DD accumulation requirement for emergence. Our chill time experiment suggests that for a 60 day chill period the thermal requirement for 50% trap catch should be 258 DD°C higher than for a 120 day chill period (see Fig. 8), which would then match the 2300 DD°C observed mean for 50% cumulative trap catch in the orchard at Red Bluff (Fig. 1). The effect of temperature on puparial development rate and thermal requirement for emergence will be repeated for a shorter chill time of 60 days in 2015 to more accurately estimate the thermal constants for WHF in California. In addition, as soil moisture appeared to lead to earlier emergence than expected in our laboratory experiments in 2014, the combined effect of temperature and 9% soil moisture will be further investigated in 2015.

Collections of WHF infested nuts from different orchards in 2013 provided some evidence for an unexpected effect of walnut cultivar on the timing of adult emergence. Adult emergence was earlier for puparia that had developed as larvae on Hartley and Vina compared to those that had developed as larvae on other cultivars. However, since two successive batches of larvae collected from the same Chandler orchard also showed some difference in emergence timing, it remains unclear the extent to which the apparent cultivar effect is real. Additional collections of larvae from different cultivars will be made in 2015 to further test for a potential interaction between cultivar and collection date.

CONCLUSION

Adult emergence of WHF is primarily driven by DD temperature accumulation. However, there was considerable variation between years at Red Bluff in the DD timing of both first and 50% cumulative trap catch, and thus other environmental factors must also play a role. Preliminary analyses suggest that more accurate estimates of the timing of first trap catch can be obtained by taking annual and mid spring rainfall into account. As expected, laboratory experiments verified that WHF puparia from California have a greater thermal requirement for adult emergence than those from Oregon. In addition, laboratory experiments showed that chill time, simulated irrigation and walnut cultivar can also influence the timing of WHF emergence and deserve further investigation.

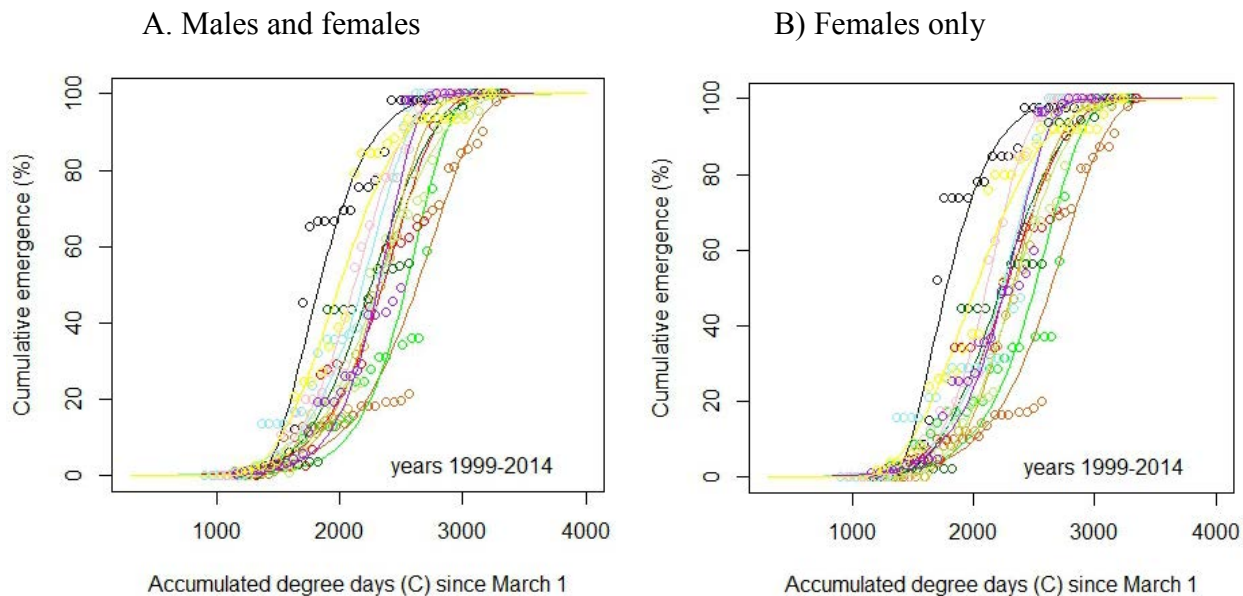


Fig. 1. The cumulative percent emergence of adult WHF in Red Bluff for selected years during the period 1999-2014 for (A) males and females combined and (B) females only.

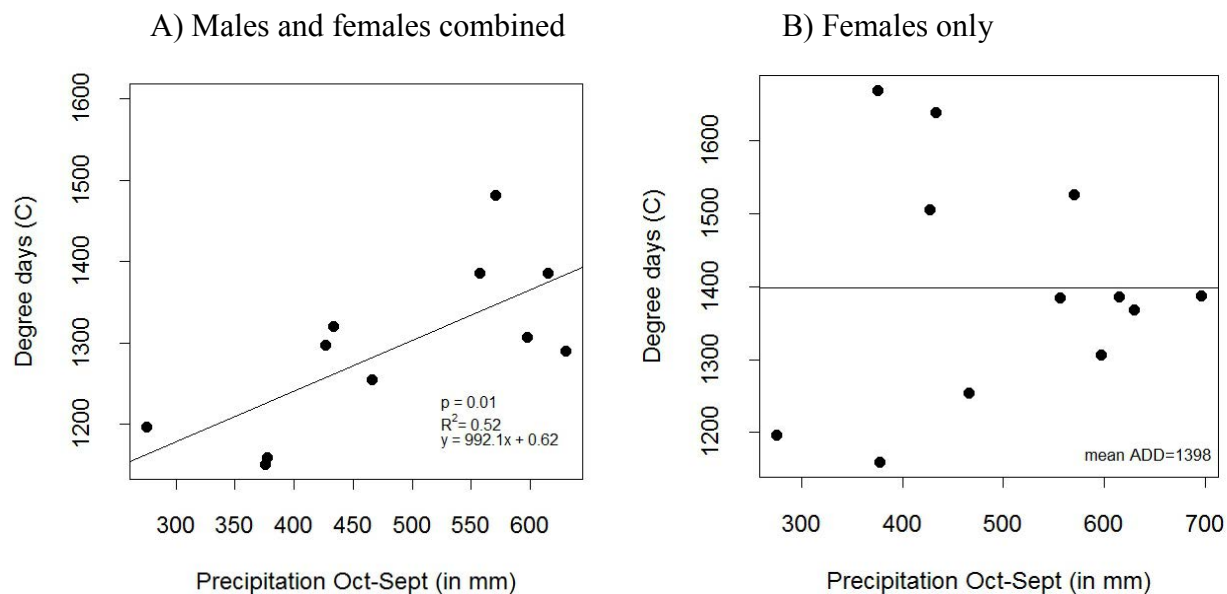


Fig. 2. Relationship between accumulated DD for first emergence of WHF adults in Red Bluff for selected years during the period 1999-2014 for (A) males and females combined and (B) females only.

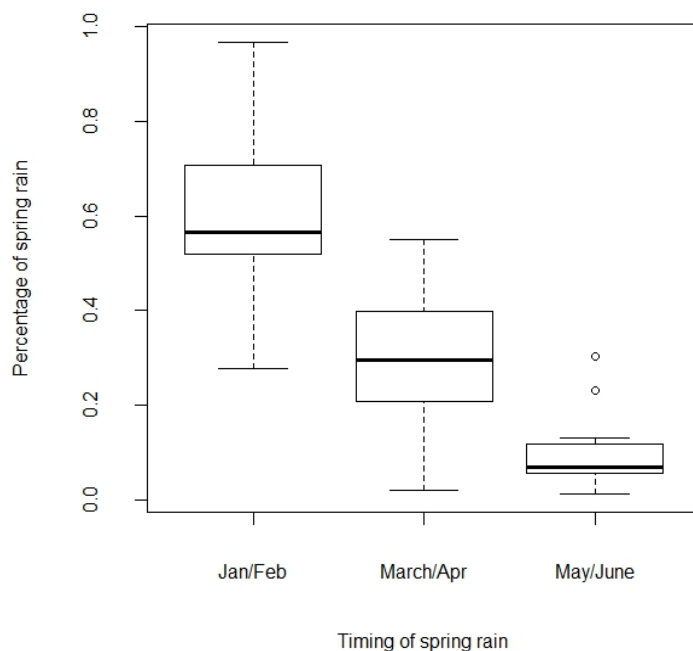


Fig. 3. Distribution of spring rainfall (January to June) in Red Bluff showing the greater variability between years in March/April compared to May/June.

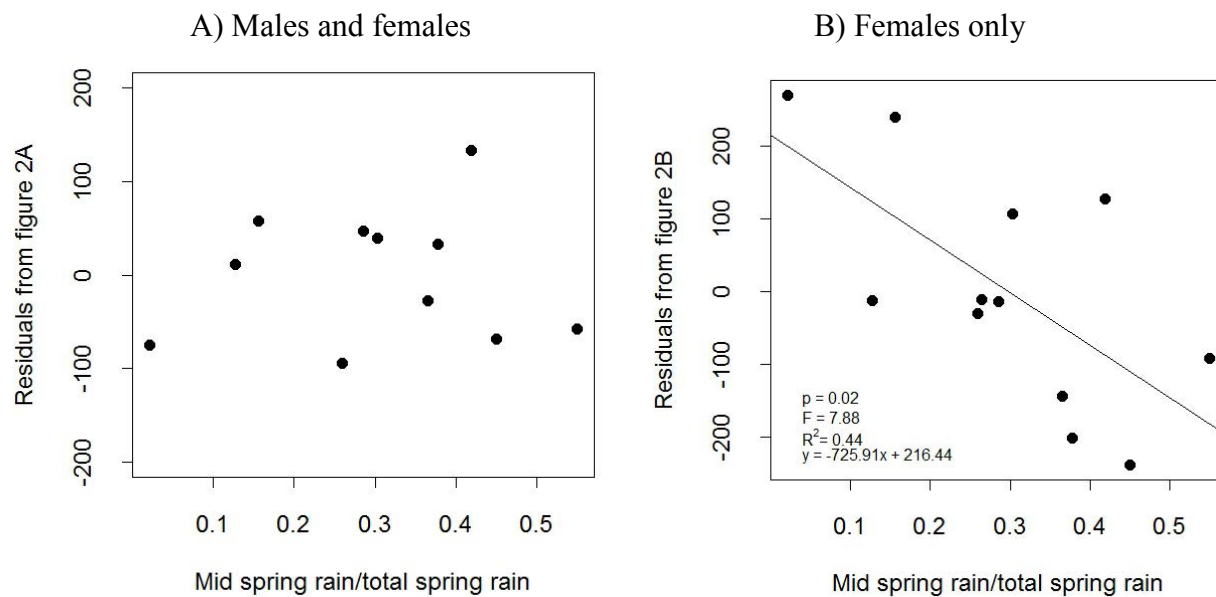


Fig. 4. The influence of mid spring rain on the residuals from the relationships between accumulated DD for first emergence of WHF for (A) males and females combined (see Fig. 2A) and (B) females only (Fig. 2B).

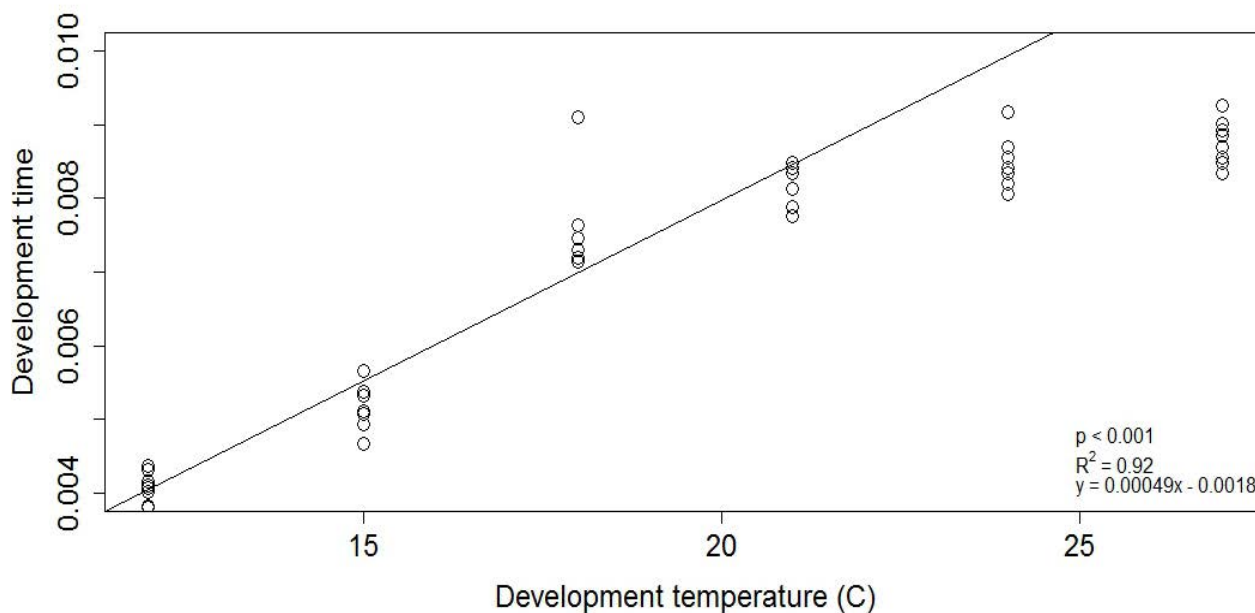


Fig. 5. Linear relationship between development rate and temperature for overwintered WHF puparia. The 24 and 27°C temperatures are plotted, but not included in the linear model.

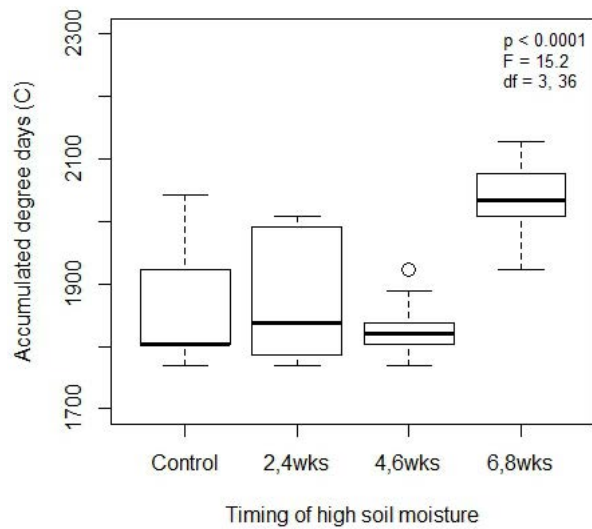


Fig. 6. The effect of timing of simulated irrigation events on the accumulated DD for median emergence of WHF adults (males and females combined).

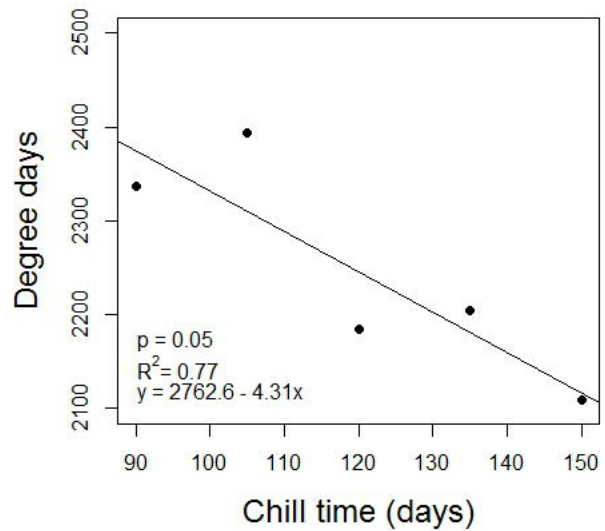


Fig. 7. The negative relationship between accumulated DD for median emergence of WHF (males and females combined) and chill time at 6 °C.

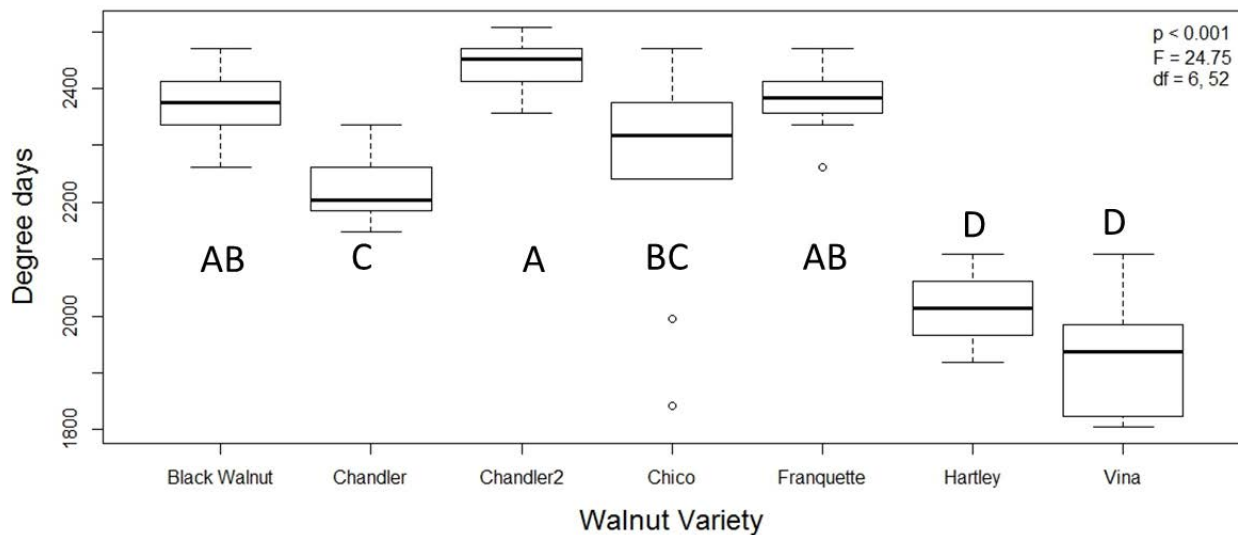


Fig. 8. The influence of walnut cultivar on the accumulated DD for median emergence of WHF adults (males and females combined).