BIOLOGY AND CONTROL OF WALNUT HUSK FLY USING REDUCED RISK PRODUCTS - 2014

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ABSTRACT

Walnuts are less susceptible to WHF infestation if they are small, have a thin husk, high trichome density and a dull dirty green hue. Chandler and Earliest have high trichome density and low WHF infestation while Serr and Payne have low trichome density and high WHF infestation. However, Hartley was an exception. Hartley had a high number of trichomes but also had a high WHF infestation. When trichomes were removed from Chandler nuts and the nuts were exposed to WHF, the infestation increased as compared to nuts with trichomes intact. Thus it appears that WHF resistance seen in Chandler is the result of several factors with trichomes being one of the factors. Trichome density appears to be stable over tree age. Thus it would be possible to determine the trichome density in mature trees when the nuts are first set. Nut color and trichome density are correlated and thus it is not necessary to count trichomes to estimate their density. GF-120 is a much better attractant/feeding stimulant than NuLure. Research is continuing on the mechanism of attraction/feeding stimulation of NuLure and other adjuvants to improve insecticide efficacy. Assail 30SG provided better larvicidal control than Admire Pro or Belay.

OBJECTIVES

Seasonal and cultivar susceptibility is known to occur with WHF infestation in walnuts. However, the factors responsible for varied susceptibility are not well understood. Mid-season leafing cultivars such as Hartley are highly susceptible while Chandler is much less susceptible. However, Chandler can become infested if there is not a more susceptible cultivar in the same orchard. Thus, simple phenological asynchrony does not appear to be a primary cause of the resistance. If the traits such as husk firmness, thickness, surface texture, chemical composition, etc. that infer resistance or susceptibility can be identified and quantified, then these traits can be used to more accurately initiate and terminate control programs and desirable characteristics can be incorporated into the breeding program. Studies were conducted to quantify nut characteristics and relate nut characteristics to WHF infestation.

Control of WHF populations has relied on the combination of a feeding stimulant and insecticide for over 50 years. The feeding stimulants and insecticides used have changed over time but the technique remains the same today as it was 50 years ago. There have been many efficacy studies comparing different insecticides with bait but few studies with temperate fruit flies (walnut husk fly, cherry fruit fly and apple maggot) showing increased efficacy with bait and mechanism of attraction. This is very surprising since various baits have been widely used by the industry over an extended time period. Thus an understanding of the mechanism of how and when feeding stimulants or attractants aid in the efficacy of WHF control would be a great benefit to the industry.

Control of WHF populations 40 years ago relied on an insecticide called phosphamidon. Phosphamidon was capable of killing adult WHF as well as penetrating the husk and killing the young larvae before significant damage occurred. Thus WHF control was highly effective, inexpensive and simple to time the applications. Then the EPA canceled the registration of phosphamidon. Replacement insecticides have been largely adulticidal with little or no larvicidal activity. Thus research over the past few years has concentrated on finding insecticides or a combination of insecticides that are both adulticidal and larvicidal and thus could be replacements for phosphamidon.

SIGNIFICANT FINDINGS

- Walnuts are less susceptible to WHF infestation if nuts are small, have a thin husk, high trichome density and a dull dirty green hue.
- Trichome density is stable with tree age and decrease during the season.
- When trichomes were removed from Chandler nuts, the WHF infestation increased.
- GF-120 (without spinosad) was a much better feeding stimulant than NuLure.
- Assail 30SG provided control of egg/larvae within the husk.

PROCEDURES

A. Identification and Quantification of Nut Characteristics and Susceptibility to WHF Infestation by Cultivar

Nut Characteristics: Ten nuts of each of nine cultivars were examined bi-weekly from 7 July to 19 August. Nuts were collected from the UC Davis variety block “Stuke Block” and transported in egg cartons to the lab at UC Berkeley for analysis. Egg cartons were used to prevent damage to the trichomes. Each nut was measured for the following parameters: trichome density, weight, husk thickness, pressure to penetrate husk, and color. Cultivars evaluated included Earliest, Hartley, Chandler, Franquette, Vina, Payne, Tulare, Carmello, and Serr. Color/reflectance was determined by using a Konica Minolta Chroma-Meter, and all nut weights were obtained using laboratory scales. Trichomes were counted under 20x magnification on a segment of husk cut from the center of the nut using a 0.7 cm diameter corer ($1.1 cm^2$). Husk thickness was measured using calipers at three places on the nut: adjacent to the stem, at mid-nut, and adjacent to the flower tip. Husk penetration pressure at the center of the nut was determined using a penetrometer fitted with a small tip (6.5 mm diameter). The pressure at puncturing the husk was recorded.

Nut Susceptibility: Eight additional nuts of each of the nine cultivars were collected and transported in egg cartons to the lab at UC Berkeley for exposure to adult WHF. One nut of each cultivar was placed in a 1 cu ft. rearing cage. There were eight rearing cages. Each nut cultivar was cut in the field with a large portion of stem still intact. The stem of each walnut was placed in a small hole in a foam plug that was placed in the opening of a 200 ml plastic Erlenmeyer flask. The flask was filled with a 3:1 water/citrus soda solution. The plug was then covered with plastic wrap and held in place with a rubber band. The soda served to increase the solution’s acidity and provide sugar for increased nut viability. The nine cultivars were placed in a randomized block design in each cage. Fifteen adult female laboratory-reared WHF were then placed in the cage. All flies used in this experiment were at least three weeks old and had been fed with a sugar/water solution and a yeast/fructose diet mixture. The yeast diet was intended to provide a protein source to promote egg production. The cages containing the cultivars also had a small amount of sugar/water solution along with regular water to sustain the flies during the experiment. After 72 hours of exposure nuts were removed. Once nuts were taken out, they were held overnight so that any late stings would darken and would be more visible. The next day, each sting was dissected to determine if eggs had been laid. This process was repeated on a biweekly basis. All data was analyzed using a Spearman Rank Order Correlation ($P \leq 0.10$).

B. Stability of Trichome Density in Chandler and Tulare Cultivars over Various Orchard Ages and Time Periods

A study was performed to compare trichome density in two walnut cultivars (Chandler and Tulare) at three different tree age categories and at three different time periods throughout the season. There were at least three different orchards within each age category and time period. Ten nuts were taken from each orchard in June, July and August. The nuts were transported in egg cartons to the lab at UC Berkeley for analysis. Egg cartons were used to prevent damage to the trichomes. Trichomes were counted under 20x magnification on a 0.7 cm diameter (1.1 cm^2) segment of husk.

C. Influence of Trichome Density on WHF Infestation

Choice and non-choice studies were conducted in the laboratory. In the non-choice study, nine Chandler nuts with trichomes removed by rubbing the nuts with unscented soap were placed in a 1 cu ft. rearing cage. Nine Chandler nuts with trichomes intact were placed in another 1 cu ft. rearing cage. There were four cages with trichomes removed and four cages with trichomes intact. Fifteen adult female laboratory-reared WHF were then placed in each of the eight cages. All flies used in this experiment were at least three weeks old and had been fed with a sugar/water solution and a yeast/fructose diet mixture. The yeast diet provided a protein source to promote egg production. The cages had a small amount of sugar/water solution and water to sustain the flies during the experiment. After 72 hours of exposure nuts were removed and held overnight so stings would darken and be more visible. The next day, each sting was dissected to determine if eggs were present. In the choice study, four nuts with trichomes removed and four nuts with trichomes intact were randomly placed in a 1 cu ft. rearing cage. There were eight cages of the two nut types. The number of flies and holding period that was used for the non-choice study was also used for the in the choice study. Data from both trials was analyzed using a two-way ANOVA (Fisher's protected LSD, $P < 0.10$).

D. Efficacy of Various Feeding Stimulant

Y-tube olfactometer studies were conducted in which two different attractants were placed in separate chambers in each arm of a Y-tube olfactometer. A known quantity of air was passed through a DRIERITE gas purifier and into the Y-tube. WHF of a known age and sex were then placed into the initial chamber of the Y-tube. Fly movement was monitored at 10-minute increments for 3 hr. This experiment was conducted with WHF ranging from 1 day to 3+ weeks of age and all male, all female, and mixed sexes. Attractants included GF-120 without spinosad, NuLure, water, sugar water, ammonium carbonate and an enzymatic yeast hydrolysate, sucrose, and vitamin WHF diet. Baits were tested at various concentrations and amounts. Trials were conducted at various airflow rates. All trials were conducted in an environmental cabinet with a constant temperature of 30˚C. A total of 29 separate treatments were tested. The experiment was terminated due to a lack of consistent and reproducible results. Choice retention studies were then conducted in which 15 male and 15 female 2-week-old WHF were placed in a 1 cu ft. rearing cage. The WHF were fed water, sugar/water and a yeast/fructose diet mixture. A 4 x 5 inch white card cut from a Pherocon 1C trap with the sticky removed was dipped into various baits and hung from the center of the cage. The cage was observed for 30 minutes and the number of WHF lands and length of time the fly remained on the card were recorded. In an initial trial, four treatments were replicated four times. The treatments were: 1:4 GF-120 (without spinosad): water, 1:4 NuLure:water, water, and an untreated control. In a second trial, four treatments were replicated six times. The treatments were: NuLure alone, 1:4 NuLure:water, 1:133 NuLure:water and water alone.

E. Ovicidal/Larvicidal Efficacy of Neonicotinoid Insecticides

Hartley walnuts were collected from the UC Davis variety block "Stuke Block" on 28 July (trial 1) and 11 August (trial 2) with a minimum of 1 to 2 inches of stem. Walnuts were immediately transported to UCB and were washed with Ivory soap and dried. The stem of each walnut was placed in a small hole in a foam plug that was placed in the opening of a 200 ml plastic Erlenmeyer flask. The flask was filled with a 3:1 water/citrus soda solution. The plug was then covered with plastic wrap and held in place with a rubber band. The last few millimeters of the stems were trimmed on the day of set-up and every 3 days after oviposition to prevent desiccation. Groups of eight walnuts were then placed in three cages with 20 female WHF per cage for one day. Female WHF had been fed on a yeast/fructose diet to promote egg production. Nuts were held for one day then dipped in experimental solutions and air-dried. The nuts were visually examined for egg hatch and larval feeding at 3, 7 and 10 days. At 10 days, the nuts were dissected to confirm the presence of larvae and instar development. The infested nuts where held at 23˚C in a constant temperature cabinet with 16:8 (L:D).

RESULTS AND DISCUSSION

A. Identification and Quantification of Nut Characteristics and Susceptibility to WHF Infestation by Cultivar

1. Nut Characteristics:

Trichome Density: Seasonal mean trichome density ranged between 301.7 and 1743.7 trichomes per cm^2 (Table 1). Carmello had the fewest number of trichomes at 301.7 trichomes per cm^2 followed by Serr and Payne at 335.3 and 335.9 trichomes per cm^2, respectively. Tulare and Vina had similar trichomes at 377.0 and 393.5 trichomes per cm^2, respectively. The number of trichomes in Franquette and Hartley increased substantially over the other cultivars at 518.3 and 583.9 trichomes per cm^2, respectively. Chandler had almost twice as many trichomes as Franquette with roughly 800.0 trichomes per cm^2 while Earliest had the highest with 1,743.7 trichomes per cm^2. It was observed with Earliest that the trichome density was highly variable with patches of extremely high trichome density while other areas had a moderate trichome density. The number of trichomes found in this study was very similar in rank order to the numbers found the previous year. However, trichome density was much higher in 2014 compared to 2013 because in 2014 a single husk cutting was taken from the middle of the nut as compared to the average of three cuttings from the stem end, middle and flower end of the nut in

2013. It was found that trichome density was lower in the stem end of the nut with the flower end intermediate between the middle of the nut and stem end of the nut.

Nut Weight: Seasonal mean nut weight ranged between 44.8 and 105.6 g (Table 2). Chandler had the lowest nut weight, at 44.8 g. Serr, Payne and Franquette had nut weights at 49.2, 49.7 and 50.0 g, respectively. Earliest, Tulare, Vina, Hartley and Carmello all had nut weights that were between 51.5 and 55.3 g. Carmello was the heaviest nut at 105.6 g. Nut weight correlated with nut size. Thus Chandler had the smallest nuts and Carmello had the largest nuts.

Husk Thickness and Penetration Pressure: Seasonal mean husk thickness at the stem end ranged between 4.9 and 6.6 mm (Table 3). The cultivar with the thinnest husk thickness was Chandler at 4.9 mm followed by Tulare at 5.7 mm. The husks of Tulare, Payne and Carmello were 6.0 mm thick while Vina, Serr and Earliest all fell in between 6.1 and 6.3 mm. Franquette had the thickest husk at the stem, with an average of 6.6 mm. The mean husk thickness mid-nut ranged between 4.4 and 5.9 mm (Table 4). Chandler had the thinnest husk thickness at mid-nut with 4.4 mm. Tulare, Earliest, Carmello and Franquette had a range of 5.0-5.3 mm. Vina, Hartley, and Serr all had husk thickness of 5.7 mm. Payne had the thickest husk at mid-nut at 5.9 mm. The mean husk thickness at the flower end ranged between 4.4 and 5.6 mm (Table 5). In most cases, the husk at the flower end of the nut was thinner than or equal to the middle or at the stem end of the nut. The cases where this was not true was with Carmello and Franquette, which were slightly larger at the flower end than at the middle of the nut. The husk penetration pressure ranged between 19.9 and 28.5 kg per cm^2 (Table 6). Earliest and Chandler produced nuts with the highest pressure required for penetration. Franquette, Carmello, Hartley and Tulare pressure ranged from 23.9 to 24.9 kg per cm^2 and fell in the middle range, while Serr, Vina and Payne were all below 23.0 kg per cm^2.

Color: Seasonal mean red to green color measurement ranged between -7.6 and -14.0. As the number becomes more negative, this represents a “greener” nut. Earliest was the least “green,” followed by Chandler (Table 7). Franquette, Payne, Vina, Hartley and Carmello had similar color measurements and fell in between -12.0 and -13.0 range. Tulare and Serr had the “greenest” nuts and measured at -13.4 and -14.0. The hue angle color measurement ranged between 105.5 and 116.2, with Earliest having the lowest hue angle measurement (Table 8). An increase in the hue angle relates to an increase in the intensity of the color. For example, on a chromaticity color diagram, a hue angle of 0 would mean that the color measurement falls within the boundaries of the most intense red color. A hue angle of 90 falls within the boundaries of the most intense yellow, and an angle of 180 is within the most intense green. Chandler, Franquette, Hartley, Payne, Vina and Carmello all fell in the middle range of measurements. Tulare and Serr had the highest hue angles, with measurements of 116.2 and 114.6.

2. Nut Susceptibility:

Trichome Density: In 2013, there was a significant negative correlation between average trichome density and the total amount of stings and stings with eggs found on each cultivar over the season. This indicated that a high trichome density might inhibit WHF infestation. This year, there was a significant correlation when comparing the total amount of the stings compared to trichome density, but not when comparing stings with egg deposition to trichome density (Tables 9 and 11). Trichome density had a stronger correlation in 2013 compared to 2014, but this does not mean that trichome density does not have any influence on infestation. During both years, Earliest, Chandler and Franquette were examples of three cultivars that had a relatively high trichome count, but a low infestation. This year, Hartley was an outlier in that Hartley had a relatively high trichome count and a very high infestation. If Hartley was viewed as an outlier and was removed from the dataset, the results were very similar to 2013 (Table 10 and 12). Nevertheless, additional studies will be required in order to confirm this year's results as well as determining why Hartley was an outlier. It is possible that Hartley's husk contains a compound(s) that acts as an attractant or that the other cultivars contain a repellent(s). Campbell et al. (1998) examined leaf volatiles of a number of species of Juglandaceae and included the cultivars Payne, Hartley and Chandler. They found a number of compounds that were present in Chandler that were absent in Payne and Hartley. They also found a number of compounds that were present in Payne and Hartley that were absent in Chandler. Thus there may be a combination of factors (trichome density and volatile or tactile constituents) that are responsible for WHF infestation.

Nut Weight: In 2013, there was a significant positive correlation between nut weight and the total amount of stings and stings with eggs found on each cultivar over the season. This was also the case for 2014. This may be due to the increased size of a nut offering increased surface area for WHF egg deposition in comparison to a smaller nut.

Husk Thickness and Penetration Pressure: In 2013, there was no significant correlation between husk thickness at the stem-end or mid-nut compared to the total amount of stings or stings with eggs found on each cultivar over the season. There was a significant positive correlation between husk thickness at the flower-end to the total amount of stings per cultivar over the season. In 2014, again there was no significant correlation between husk thickness at the stem-end compared to the total amount of stings found on each cultivar over the season. However, there was a significant positive correlation between husk thickness at mid-nut and at flower-end. There was no correlation between husk thickness and the total amount of stings with egg deposition. The significant positive correlation between infestation and flower-end of the nut could be an artifact of the study. The nuts were placed flower-end up in the oviposition cages and the flower-end received greater exposure to the flies. Thus there was a significant preference for WHF to oviposit into thick husk. In both 2013 and 2014, there was no significant correlation between husk penetration pressure and the total amount of stings or stings with eggs found on any cultivar over the season.

Color: In 2013 and 2014, there was a significant negative correlation between the mean red to green color range measurement and the total number of stings and stings with eggs found on each cultivar over the season. The more negative the red to green color range measurement, the greater the amount of "greenness" of a cultivar. WHF have been shown to be highly selective for various shades of green. Riedl et al. (1981) found significantly higher trap catch with traps that were Shamrock or Foliage green as compared to light green. Also when Hartley cultivar was removed from the analysis the probability value improved both for red/green color and hue. In 2013, there was also a significant positive correlation between the mean hue angle measurement and stings, but not with the total amount of stings with eggs found on each cultivar over the season. In 2014, this was reversed, and there was a positive correlation between egg deposition and hue angle but not total WHF stings. The positive correlation showed that a larger hue angle in nut husks correlates with increased susceptibility to WHF infestation. In this case, as the hue angle gets closer to 180, the intensity of the green color increases. Therefore, nuts that have a brighter green color are more attractive than dull pale green nuts. The increase in the trichome density may cause the nuts to have duller color.

B. Stability of Trichome Density in Chandler and Tulare Cultivars over Various Orchard Ages and Time Periods

Trichome density Chandler and Tulare cultivars on nuts from trees of various ages were not significantly different (Tables 13 and 14). However both cultivars showed a decreasing density of trichome as the season progressed from June to August. This decrease could be the result of various abrasive environmental factors or simply a dilution as a result of nut surface growth. However, trichome density is stable with tree from 3 to 25 years to age. Thus trichome density on nuts of mature trees can be determined from nuts of trees that are only three or four years old.

C. Influence of Trichome Density on WHF Infestation

In the WHF susceptibility study, the Chandler cultivar had very low WHF infestation, as well as a high number of trichomes. Therefore, Chandler was an ideal cultivar to use for this experiment. In the no-choice study, the mean number of WHF stings increased when the trichomes were removed from 0.71 stings per nut with trichomes to 1.0 stings per nut without trichomes (Table 15). However, the increase in stings was not significantly different with a P value of 0.4. Thus, when WHF females have no choice they will oviposit on nuts with trichome. In the choice study, the mean number of WHF stings increased from 0.91 stings per nut when the trichomes were not removed to 1.31 stings per nut without trichomes. The increase in stings was significantly different with a P value of 0.1. This study indicates that trichomes can be an influencing factor in WHF oviposition. However, it appears that it is not the only factor.

D. Efficacy of Various Feeding Stimulants

Repeated experiments with the Y-tube olfactometer did not produce any meaningful results and the experiment was abandoned and a simple observational study was conducted. In the first trial, the untreated check had significantly greater number of WHF landing on the target compared to 1:4 GF-120 without spinosad: water, 1:4 NuLure: water or water alone (Table 17). However, total time the flies remained on the target and the amount of time an individual fly remained on the target was significantly longer in 1:4 GF-120 without spinosad: water as compared to the other treatments. Thus was indicated that once a fly landed on the 1:4 GF-120 without spinosad: water target it remained on the target. Also, there was no significant difference in the total time or time per visit between water and 1:4 NuLure: water. This is a very surprising result that needs further investigation. In the second trial, 3:400 NuLure: water had significantly greater number of WHF lands on the target compared to pure NuLure and water (Table 18). However, total time the flies remained on the target was significantly longer in water and 1:133 NuLure: water compared to the other treatments. The amount of time an individual fly remained on the target was significantly longer in water alone and the time decreased with the amount of water. This would indicate that the flies were seeking water more than the NuLure. Again, this is a very surprising result that needs further investigation.

E. Ovicidal/Larvicidal Efficacy of Neonicotinoid Insecticides

In a trial with the highest registered rate of neonicotinoid insecticides, only Assail provided significant control while Admire Pro showed some suppression of WHF infestation (Table 19). Assail 30SG resulted in nearly complete egg/larvae mortality through presumably translaminar movement of the insecticide into the walnut husk. Admire Pro resulted in minor egg/larvae mortality, while both Belay and the untreated check provided no control of WHF. Since the high rate of Assail 30SG provided significant control the experiment was repeated with the lowest registered rate of Assail. Again Assail showed some measure of WHF larval infestation (Table 20). Chi-square analysis of both trials indicated that there was significant difference ($P \leq 0.05$) in first trial but not in the second trial.

When comparing these results to the previous year's trials, there is a notable difference in WHF control. In the 2013 trials, both the imidacloprid (Provado) and clothianidin (Belay) solutions provided significant control of WHF infestation. However, this was not the case in this year's trials. This could be a result of WHF health and the viability of the eggs that were laid in the tested walnuts. Last year's WHF were fed on a diet solely of sugar and water, and took a week to generate the minimum two stings per nut required for the experiment. This year, the addition of a protein (yeast) seemed to increase the health and production of the flies, and they only needed one day to reach the two-sting minimum.

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Table 1. Mean number of trichomes per square centimeter per cultivar at various dates, Davis, CA – 2014

Cultivar	Mean number trichomes/cm^2				
	8 Jul	22 Jul	5 Aug	19 Aug	Season
Earliest	1472.0 ± 149.8	1920.0 ± 229.1	1708.2 ± 139.2	1874.5 ± 117.8	1743.7 ± 101.3
Hartley	583.6 ± 20.1	623.4 ± 59.8	496.8 ± 44.9	631.9 ± 17.7	583.9 ± 30.9
Chandler	798.0 ± 19.5	807.3 ± 21.8	800.8 ± 28.5	793.8 ± 26.8	800.0 ± 2.8
Franquette	532.2 ± 23.3	543.3 ± 22.1	538.7 ± 29.6	459.1 ± 34.7	518.3 ± 19.9
Vina	434.5 ± 18.7	415.2 ± 38.3	327.1 ± 24.2	397.0 ± 30.6	393.5 ± 23.4
Payne	361.7 ± 23.8	329.2 ± 23.0	266.1 ± 29.8	386.6 ± 25.1	335.9 ± 26.1
Tulare	353.9 ± 21.1	411.3 ± 32.2	380.9 ± 23.2	361.7 ± 22.3	377.0 ± 12.8
Carmello	260.1 ± 15.7	343.3 ± 22.4	318.1 ± 25.4	285.6 ± 19.5	301.7 ± 18.2
Serr	360.9 ± 28.6	336.2 ± 21.5	305.8 ± 23.8	338.1 ± 24.5	335.3 ± 11.3

Table 2. Mean nut weight (g) per cultivar at various dates, Davis, CA – 2014

Cultivar	Mean nut weight (g)				
	7 Jul	21 Jul	4 Aug	18 Aug	Season
Earliest	46.7 ± 1.9	52.9 ± 1.7	54.6 ± 1.1	51.9 ± 3.4	51.5 ± 1.7
Hartley	52.0 ± 2.6	54.5 ± 2.4	55.5 ± 2.7	59.1 ± 1.7	55.3 ± 1.5
Chandler	40.2 ± 2.9	45.2 ± 2.7	47.7 ± 1.2	46.3 ± 2.5	44.8 ± 1.6
Franquette	45.2 ± 2.0	50.9 ± 2.1	60.4 ± 4.0	43.5 ± 2.8	50.0 ± 3.8
Vina	51.6 ± 1.7	54.2 ± 2.3	47.3 ± 4.2	59.7 ± 4.9	53.2 ± 2.6
Payne	46.6 ± 2.6	54.5 ± 1.9	51.4 ± 2.5	46.4 ± 1.8	49.7 ± 2.0
Tulare	53.1 ± 1.1	48.6 ± 1.8	49.7 ± 1.8	55.9 ± 2.6	51.8 ± 1.7
Carmello	98.0 ± 6.0	109.7 ± 4.8	103.6 ± 6.5	110.9 ± 8.1	105.6 ± 3.0
Serr	43.3 ± 0.6	52.7 ± 1.8	53.0 ± 2.9	47.9 ± 1.7	49.2 ± 2.3

Table 3. Mean husk thickness (mm) at stem-end per cultivar at various dates, Davis, CA – 2014

Cultivar	Mean husk thickness (mm) at stem-end				
	7 Jul	21 Jul	4 Aug	18 Aug	Season
Earliest	5.9 ± 0.2	7.2 ± 0.4	6.2 ± 0.3	6.1 ± 0.3	6.3 ± 0.3
Hartley	5.9 ± 0.2	4.9 ± 0.3	6.5 ± 0.4	6.5 ± 0.2	6.0 ± 0.4
Chandler	4.7 ± 0.3	5.6 ± 0.3	4.8 ± 0.1	4.5 ± 0.3	4.9 ± 0.2
Franquette	5.6 ± 0.5	7.4 ± 0.2	6.9 ± 0.3	6.3 ± 0.3	6.6 ± 0.4
Vina	6.6 ± 0.3	6.5 ± 0.3	6.0 ± 0.3	5.3 ± 0.4	6.1 ± 0.3
Payne	5.8 ± 0.2	6.3 ± 0.3	6.3 ± 0.3	5.5 ± 0.4	6.0 ± 0.2
Tulare	5.8 ± 0.3	5.9 ± 0.3	5.6 ± 0.3	5.5 ± 0.4	5.7 ± 0.1
Carmello	4.5 ± 0.3	7.3 ± 0.2	6.0 ± 0.3	6.0 ± 0.4	6.0 ± 0.6
Serr	5.6 ± 0.3	7.1 ± 0.2	5.9 ± 0.2	6.2 ± 0.3	6.2 ± 0.3

Table 4. Mean husk thickness (mm) at mid-nut per cultivar at various dates, Davis, CA – 2014.

Cultivar	Mean husk thickness (mm) at mid-nut				
	7 Jul	21 Jul	4 Aug	18 Aug	Season
Earliest	4.8 ± 0.3	5.7 ± 0.3	5.1 ± 0.3	4.9 ± 0.3	5.1 ± 0.2
Hartley	5.3 ± 0.3	5.3 ± 0.5	6.2 ± 0.4	6.1 ± 0.2	5.7 ± 0.3
Chandler	4.3 ± 0.2	5.2 ± 0.3	4.5 ± 0.1	3.6 ± 0.3	4.4 ± 0.3
Franquette	4.1 ± 0.3	5.9 ± 0.3	6.0 ± 0.3	5.2 ± 0.4	5.3 ± 0.4
Vina	5.5 ± 0.2	5.7 ± 0.4	6.1 ± 0.4	5.3 ± 0.2	5.7 ± 0.2
Payne	5.6 ± 0.3	5.7 ± 0.2	6.3 ± 0.3	5.9 ± 0.5	5.9 ± 0.2
Tulare	4.9 ± 0.2	5.9 ± 0.3	5.0 ± 0.2	4.3 ± 0.2	5.0 ± 0.3
Carmello	3.7 ± 0.3	6.2 ± 0.1	5.9 ± 0.2	4.9 ± 0.2	5.2 ± 0.6
Serr	5.6 ± 0.3	6.0 ± 0.3	5.8 ± 0.3	5.6 ± 0.3	5.7 ± 0.1

Table 5. Mean husk thickness (mm) at flower-end per cultivar at various dates, Davis, CA – 2014.

	Mean husk thickness (mm) at flower-end				
Cultivar	7 Jul	21 Jul	4 Aug	18 Aug	Season
Earliest	5.2 ± 0.3	4.9 ± 0.2	5.0 ± 0.2	5.4 ± 0.3	5.1 ± 0.1
Hartley	5.1 ± 0.3	5.4 ± 0.2	5.8 ± 0.3	6.0 ± 0.2	5.6 ± 0.2
Chandler	4.8 ± 0.3	4.9 ± 0.1	4.2 ± 0.1	3.7 ± 0.2	4.4 ± 0.3
Franquette	4.7 ± 0.3	6.3 ± 0.2	6.2 ± 0.3	4.7 ± 0.2	5.5 ± 0.4
Vina	5.5 ± 0.2	5.5 ± 0.2	5.5 ± 0.4	5.2 ± 0.3	5.4 ± 0.1
Payne	5.5 ± 0.3	5.7 ± 0.3	5.8 ± 0.2	5.5 ± 0.4	5.6 ± 0.1
Tulare	5.1 ± 0.2	5.1 ± 0.3	4.9 ± 0.2	3.9 ± 0.2	4.7 ± 0.3
Carmello	4.3 ± 0.2	6.4 ± 0.2	6.2 ± 0.2	4.4 ± 0.2	5.3 ± 0.6
Serr	5.1 ± 0.2	5.5 ± 0.2	5.3 ± 0.2	5.0 ± 0.3	5.2 ± 0.1

Table 6. Mean pressure to penetrate (kg/cm^2) per cultivar at various dates, Davis, CA – 2014.

	Mean pressure to penetrate (kg/cm^2)				
Cultivar	7 Jul	21 Jul	4 Aug	18 Aug	Season
Earliest	32.7 ± 1.3	28.7 ± 1.1	28.5 ± 1.0	24.0 ± 0.6	28.5 ± 1.8
Hartley	25.3 ± 0.8	23.4 ± 1.1	25.7 ± 0.7	21.8 ± 1.0	24.0 ± 0.9
Chandler	30.9 ± 1.1	28.1 ± 1.1	25.5 ± 0.6	25.5 ± 1.3	27.5 ± 1.3
Franquette	25.9 ± 1.0	23.3 ± 0.7	22.8 ± 1.1	23.7 ± 1.3	23.9 ± 0.7
Vina	24.0 ± 1.1	20.6 ± 0.7	22.7 ± 1.2	17.7 ± 1.0	21.3 ± 1.4
Payne	23.5 ± 1.5	21.4 ± 0.8	23.5 ± 0.6	20.0 ± 1.3	22.1 ± 0.9
Tulare	26.7 ± 1.1	25.3 ± 0.9	24.1 ± 1.0	23.3 ± 0.9	24.9 ± 0.8
Carmello	25.3 ± 1.1	24.7 ± 1.4	23.7 ± 1.1	22.2 ± 0.7	24.0 ± 0.7
Serr	24.3 ± 1.1	19.0 ± 0.7	19.3 ± 0.7	16.8 ± 1.1	19.9 ± 1.6

Table 7. Mean red to green color measurement per cultivar at various dates, Davis, CA – 2014

	Mean red to green color measurement				
Cultivar	7 Jul	21 Jul	4 Aug	18 Aug	Season
Earliest	-8.3 ± 1.0	-7.1 ± 0.9	-6.3 ± 0.7	-8.8 ± 0.6	-7.6 ± 0.6
Hartley	-13.1 ± 0.1	-12.4 ± 0.1	-12.3 ± 0.2	-12.5 ± 0.2	-12.6 ± 0.2
Chandler	-12.7 ± 0.2	-11.5 ± 0.2	-11.2 ± 0.3	-11.3 ± 0.2	-11.7 ± 0.3
Franquette	-13.1 ± 0.1	-11.9 ± 0.1	-11.7 ± 0.1	-11.4 ± 0.2	-12.0 ± 0.4
Vina	-12.8 ± 0.2	-12.6 ± 0.2	-12.2 ± 0.2	-12.2 ± 0.1	-12.4 ± 0.2
Payne	-13.2 ± 0.2	-11.8 ± 0.1	-12.0 ± 0.2	-11.7 ± 0.3	-12.2 ± 0.4
Tulare	-13.7 ± 0.2	-13.4 ± 0.3	-13.4 ± 0.2	-12.9 ± 0.3	-13.4 ± 0.2
Carmello	-13.1 ± 0.2	-12.4 ± 0.3	-12.7 ± 0.3	-13.1 ±0.3	-12.8 ± 0.2
Serr	-14.7 ± 0.2	-13.4 ± 0.2	-13.9 ± 0.2	-14.2 ± 0.3	-14.0 ± 0.3

Table 8. Mean hue angle color measurement per cultivar at various dates, Davis, CA – 2014

	Mean hue angle color measurement				
Cultivar	7 Jul	21 Jul	4 Aug	18 Aug	Season
Earliest	106.7 ± 1.7	105.0 ± 1.9	103.1 ± 1.3	107.1 ± 1.0	105.5 ± 0.9
Hartley	113.2 ± 0.3	112.9 ± 0.3	112.1 ± 0.3	112.6 ± 0.3	112.7 ± 0.2
Chandler	112.0 ± 0.4	112.4 ± 0.4	111.7 ± 0.5	111.9 ± 0.4	112.0 ± 0.2
Franquette	113.3 ± 0.2	112.9 ± 0.3	112.7 ± 0.3	111.5 ± 0.3	112.6 ± 0.4
Vina	113.8 ± 0.2	114.3 ± 0.2	112.2 ± 0.4	112.2 ± 0.3	113.1 ± 0.6
Payne	113.6 ± 0.3	113.1 ± 0.4	113.3 ± 0.4	111.9 ± 0.4	113.0 ± 0.4
Tulare	116.8 ± 0.2	116.5 ± 0.5	116.4 ± 0.4	115.3 ± 0.3	116.2 ± 0.3
Carmello	114.1 ± 0.4	114.4 ± 0.3	114.7 ± 0.3	113.9 ± 0.3	114.3 ± 0.2
Serr	114.7 ± 0.2	115.6 ± 0.3	114.0 ± 0.4	114.3 ± 0.3	114.6 ± 0.4

Table 9. Correlations between total WHF stings and walnut characteristics by cultivar - 2014

		Mean over entire season							
	Total	Trichomes	Weight	Husk thickness (mm)			Husk penetration	Color	
Cultivar	stings	per cm^2	(g)	Stem-end	Mid-nut	Flower-end	per cm^2	Red/green	Hue angle
Earliest	46	1743.7	51.50	6.31	5.14	5.15	28.49	-7.62	105.49
Hartley	106	583.9	55.26	5.96	5.73	5.58	24.04	-12.59	112.69
Chandler	29	800.0	44.84	4.90	4.40	4.41	27.51	-11.67	112.02
Franquette	46	518.3	49.97	6.56	5.32	5.46	23.89	-12.02	112.59
Vina	59	393.5	53.20	6.09	5.66	5.41	21.26	-12.45	113.13
Payne	64	335.9	49.70	5.97	5.87	5.60	22.11	-12.17	112.97
Tulare	61	377.0	51.83	5.69	5.03	4.74	24.85	-13.37	116.24
Carmello	65	301.7	105.55	5.96	5.18	5.31	23.97	-12.82	114.26
Serr	62	335.3	49.22	6.19	5.73	5.21	19.86	-14.05	114.63
Correlation Coefficient[a]		**-0.594**	**0.561**	-0.185	**0.630**	**0.569**	-0.343	**-0.653**	0.519
P Value		**0.077**	**0.099**	0.612	**0.058**	**0.099**	0.331	**0.050**	0.138

[a] Correlations in bold are statistically significant (P<0.10) using a Spearman Rank Correlation

Table 10. Correlations between total WHF stings and walnut characteristics (without Hartley) by cultivar - 2014

		Mean over entire season							
	Total	Trichomes	Weight	Husk thickness (mm)			Husk penetration	Color	
Cultivar	stings	per cm^2	(g)	Stem-end	Mid-nut	Flower-end	per cm^2	Red/green	Hue angle
Earliest	46	1743.7	51.50	6.31	5.14	5.15	28.49	-7.62	105.49
Chandler	29	800.0	44.84	4.90	4.40	4.41	27.51	-11.67	112.02
Franquette	46	518.3	49.97	6.56	5.32	5.46	23.89	-12.02	112.59
Vina	59	393.5	53.20	6.09	5.66	5.41	21.26	-12.45	113.13
Payne	64	335.9	49.70	5.97	5.87	5.60	22.11	-12.17	112.97
Tulare	61	377.0	51.83	5.69	5.03	4.74	24.85	-13.37	116.24
Carmello	65	301.7	105.55	5.96	5.18	5.31	23.97	-12.82	114.26
Serr	62	335.3	49.22	6.19	5.73	5.21	19.86	-14.05	114.63
Correlation Coefficient[a]		**-0.934**	0.395	-0.132	0.551	0.431	-0.479	**-0.695**	**0.671**
P Value		**0.000**	0.290	0.705	0.139	0.260	0.207	**0.047**	**0.059**

[a] Correlations in bold are statistically significant (P<0.10) using a Spearman Rank Correlation

Table 11. Correlations between WHF egg depositions and walnut characteristics by cultivar - 2014

		Mean over entire season							
	Total stings	Trichomes	Weight	Husk thickness (mm)			Husk penetration	Color	
Cultivar	w/ eggs	per cm^2	(g)	Stem-end	Mid-nut	Flower-end	per cm^2	Red/green	Hue angle
Earliest	39	1743.7	51.50	6.31	5.14	5.15	28.49	-7.62	105.49
Hartley	89	583.9	55.26	5.96	5.73	5.58	24.04	-12.59	112.69
Chandler	24	800.0	44.84	4.90	4.40	4.41	27.51	-11.67	112.02
Franquette	39	518.3	49.97	6.56	5.32	5.46	23.89	-12.02	112.59
Vina	55	393.5	53.20	6.09	5.66	5.41	21.26	-12.45	113.13
Payne	54	335.9	49.70	5.97	5.87	5.60	22.11	-12.17	112.97
Tulare	57	377.0	51.83	5.69	5.03	4.74	24.85	-13.37	116.24
Carmello	59	301.7	105.55	5.96	5.18	5.31	23.97	-12.82	114.26
Serr	54	335.3	49.22	6.19	5.73	5.21	19.86	-14.05	114.63
Correlation Coefficient[a]		-0.471	**0.824**	-0.304	0.333	0.361	-0.193	**-0.698**	**0.613**
P Value		0.186	**0.004**	0.407	0.356	0.308	0.580	**0.030**	**0.067**

[a] Correlations in bold are statistically significant (P<0.10) using a Spearman Rank Correlation

Table 12. Correlations between WHF egg depositions and walnut characteristics (without Hartley) by cultivar - 2014

		Mean over entire season							
	Total stings	Trichomes	Weight	Husk thickness (mm)			Husk penetration	Color	
Cultivar	w/ eggs	per cm^2	(g)	Stem-end	Mid-nut	Flower-end	per cm^2	Red/green	Hue angle
Earliest	39	1743.7	51.50	6.31	5.14	5.15	28.49	-7.62	105.49
Chandler	24	800.0	44.84	4.90	4.40	4.41	27.51	-11.67	112.02
Franquette	39	518.3	49.97	6.56	5.32	5.46	23.89	-12.02	112.59
Vina	55	393.5	53.20	6.09	5.66	5.41	21.26	-12.45	113.13
Payne	54	335.9	49.70	5.97	5.87	5.60	22.11	-12.17	112.97
Tulare	57	377.0	51.83	5.69	5.03	4.74	24.85	-13.37	116.24
Carmello	59	301.7	105.55	5.96	5.18	5.31	23.97	-12.82	114.26
Serr	54	335.3	49.22	6.19	5.73	5.21	19.86	-14.05	114.63
Correlation Coefficient[a]		**-0.759**	**0.771**	-0.253	0.205	0.193	-0.313	**-0.747**	**0.807**
P Value		**0.021**	**0.021**	0.498	0.578	0.619	0.423	**0.029**	**0.010**

[a] Correlations in bold are statistically significant (P<0.10) using a Spearman Rank Correlation

Table 13. Mean number of trichomes per square centimeter per nut at three time periods for various ages of Chandler cultivar – 2014

Orchard age	No. of orchards	Mean[a] no. trichomes per square centimeter		
		June	July	August
3-4 years	4	382 a	342 a	298 a
10-11 years	4	343 a	346 a	296 a
19-26 years	4	367 a	351 a	287 a

[a] Means followed by the same letter in a column were not significantly different (means separated using Fisher's LSD at $P \leq 0.05$).

Table 14. Mean number of trichomes per square centimeter at three time periods for various ages of Tulare cultivar – 2014

Orchard age	No of orchards	Mean[a] no. trichomes per square centimeter		
		June	July	August
4-8 years	4	257 a	173 a	203 a
10-14 years	3	242 a	192 a	195 a
18-25 years	3	218 a	160 a	207 a

[a] Means followed by the same letter in a column were not significantly different (means separated using Fisher's LSD at $P \leq 0.05$).

Table 15. Mean number of WHF stings per nut and percent stings with and without eggs in nuts with and without trichomes in a non-choice test – 2014

Treatment	Mean[a] no. stings	Mean[a] percent stings without eggs	Mean[a] percent stings with eggs
Trichomes present	0.71 a	20 a	80 a
Trichomes removed	1.00 a	17 a	83 a

[a] Means followed by the same letter in a column were not significantly different (means separated using Fisher's LSD at $P \leq 0.05$).

Table 16. Mean number of WHF stings per nut and percent stings with and without eggs in nuts with and without trichomes in a choice test – 2014

Treatment	Mean[a] no. stings	Mean[a] percent stings without eggs	Mean[a] percent stings with eggs
Trichomes present	0.91 a	14 a	86 a
Trichomes removed	1.31 b	7 a	93 a

[a] Means followed by the same letter in a column were not significantly different (means separated using Fisher's LSD at $P \leq 0.1$).

Table 17. Mean WHF landings, total retention time and retention time per landing to dilute GF-120, dilute NuLure and water, 2014

Treatment	Rate.	Mean[a] No. lands	Mean[a] total time	Mean[a] time/land
GF-120:water	1:4	21.3 c	4594.3 a	226.2 a
NuLure:water	1:4	34.0 b	873.0 bc	23.5 b
Water	--	38.5 b	1974.5 b	49.0 b
Untreated check	--	61.0 a	385.5 c	7.0 b

[a] Means followed by the same letter in a column were not significantly different (means separated using Fisher's LSD at $P \leq 0.05$).

Table 18. Mean WHF landings, total retention time and retention time per landing to various concentrations of dilute NuLure and water alone, 2014

Treatment	Rate	Mean[a] No. lands	Mean[a] total time	Mean[a] time/land
NuLure:water	1:0	27.8 c	331.3 b	10.2 c
NuLure:water	1:4	44.7 ab	773.5 b	15.7 bc
NuLure:water	3:400	49.7 a	1558.0 a	31.5 b
Water	--	35.3 c	1806.5 a	52.1 a

[a] Means followed by the same letter in a column were not significantly different (means separated using Fisher's LSD at $P \leq 0.05$).

Table 19. Mean percent nuts with larvae and mean number of stings per nut, trial 1—2014

Treatment:	Rate Form/100 gal	Mean number Stings per nut	Mean percent nuts with larvae
Admire Pro[a]	2.8 fl oz	2.8	83.3
Assail 30SG[a]	9.6 oz	3.0	33.3
Belay 2.13SC[a]	6.0 fl oz	2.8	100.0
Untreated check[a]	-----	2.8	100.0

[a] Included 0.0125% v/v of MSO

Table 20. Mean percent nuts with larvae and mean number of stings per nut, trial 2—2014

	Rate Form/100 gal	Mean number stings per nut	Mean percent nuts with larvae
Assail 30SG[a]	6.4 oz	2.8	66.6
Untreated check[a]	-----	2.8	100.0

[a] Included 0.0125% v/v of MSO.