

FINAL REPORT
WTFREC Project #AE-02-220

WSU Project #13C-3643-4386

Project title: Feeding behavior, thresholds, and pheromone trapping of *Campylomma verbasci*

PI: Elizabeth H. Beers, Entomologist
Organization: WSU Tree Fruit Research and Extension Center, Wenatchee
(509) 663-8181 ext 234 ebeers@wsu.edu

Co-PIs and affiliations: Stephen D. Cockfield, Research Associate,
WSU Tree Fruit Research and Extension Center, Wenatchee

Contract administrator: Mary Lou Bricker (mldesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu); (509) 335-3691

Objectives:

1. Modify and validate fall pheromone trap sampling as a method of identifying high-risk orchards for spring sampling ('Delicious' and 'Golden Delicious').
2. Determine the relative susceptibility to *C. verbasci* damage of apple cultivars other than 'Delicious' and 'Golden Delicious'.
3. Develop provisional treatment thresholds on susceptible apple cultivars that currently have none.

Significant findings:

1. Pheromone trapping of adult *C. verbasci* in the fall was successful in determining risk of nymph infestation the following spring.
 - ◇ The Pherocon IV and the smaller Pherocon II trap caught significantly fewer *C. verbasci* than did the nearly obsolete Pherocon 1C, and the efficiency of the Pherocon IV was approximately half that of the 1C. However, capture in the Pherocon IIB trap was not significantly different from that of the 1C.
 - ◇ Risk assessments were most reliable in 'Delicious' orchards. All the orchards determined to be low risk had undetectable nymph populations. In one case, traps gave advance warning of an extremely severe spring infestation. All errors were false positives.
 - ◇ Risk assessments were less reliable in 'Golden Delicious' orchards. Although most low trap catches resulted in below-threshold nymph populations, injury was often evident at harvest. Because of the high sensitivity of 'Golden Delicious', there was an inherent problem in the reliability of tap samples (all errors, 5/16 observations, were false negatives).
2. Most common cultivars are susceptible to *C. verbasci* injury.
 - ◇ In field assays evaluated before June, the susceptibility of 'Gala', 'Granny Smith', 'Fuji', and 'Cameo' to feeding injury appeared to be intermediate between that of 'Delicious' and 'Golden Delicious'.
 - ◇ 'Braeburn' appears highly resistant to *C. verbasci*.
3. The economic injury level for *C. verbasci* on 'Gala' is ca.1 nymph per tap. In 2003 and 2004, about half to two-thirds of the injury on 'Gala' was minute and would not cause culling. Some injury appeared atypical (russet or tiny dark spots), and some typical (a combination of bumps and dimples).

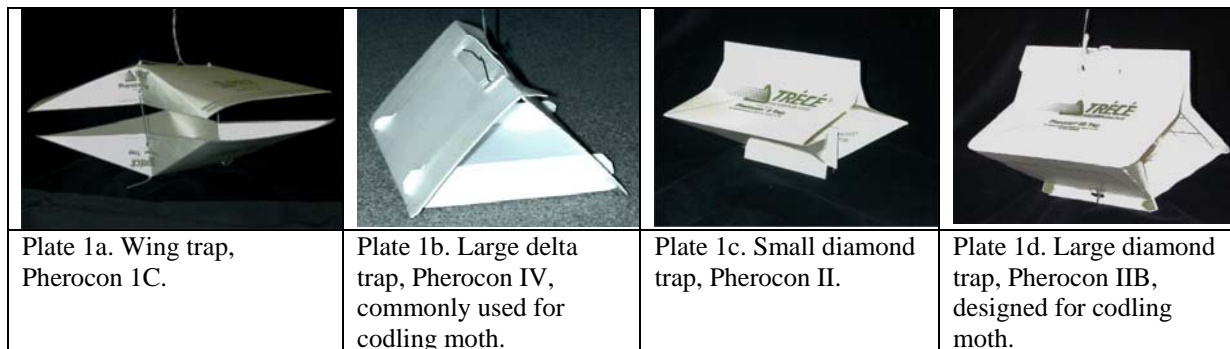
Methods:

Validation of pheromone trap sampling:

Comparison of pheromone trap designs: Beginning in August, two Pherocon 1C traps and two Pherocon IV traps loaded with *C. verbasci* pheromone flex lures (Phero-Tech, LTD) were placed in

four 10-acre blocks (replicates) in Brewster, WA. Traps were checked weekly. Each trap station, four per block, was at the center of a 2.5-acre quadrant. Individual traps were re-randomized among the four trap stations after each weekly count. Traps were collected after 5 weeks (the recommended lure replacement interval). Data analysis consisted of an analysis of variance with LSD mean separation for the total number of males captured per trap over the 5-week study.

Four trap designs (Pherocon 1C, Pherocon IV, Pherocon II, and Pherocon IIB) (Trécé, Inc.) were compared in an Orondo orchard with a high population of *C. verbasci* adults. Four replicate blocks of the four trap types were placed in a single row of the orchard. Traps within blocks were placed every other tree, and blocks were separated by 10 trees. After 1 week, the number of males caught in the Pherocon IV, II, and IIB traps was compared to the number caught in the 1C traps (the current standard). Differences in trap catch were tested for significance with Dunnett's test.



Validation of risk thresholds: Ten 'Delicious' and 10 'Golden Delicious' orchards with a history of *C. verbasci* damage were selected in north-central Washington to serve as test subjects of the fall pheromone trapping technique. Each 10-acre block was divided into four 2.5-acre quadrants. Pheromone trap procedures followed those of Reding (2000). One Pherocon IV trap was placed 2 m above the ground in the center of each quadrant. *C. verbasci* pheromone lures were suspended in the center of each trap. Trap liners and lures were changed every 4-6 weeks from the last week of July to the first week of November. The risk of encountering a high spring population of nymphs was calculated according to the thresholds modified for the large delta trap (see Results and Discussion). We based our thresholds on those proposed by Reding (2000) for the "long-fall" trapping period in untreated orchards. Of his thresholds offered, one set for commercial and one set for untreated orchards, the ones for untreated orchards are the most conservative. The delta-trap threshold was 175 nymphs per trap for 'Delicious' and 125 for 'Golden Delicious'. Densities of secondary pests that serve as prey for *C. verbasci* were assessed in November. In 2002, about 25 spurs were randomly selected and examined under a microscope for European red mite (ERM) eggs. One hundred shoots were examined in the field for signs of aphid colonies (aphid parts, honeydew, or sooty mold). In 2003, 100 spurs and 50 shoots were inspected from each block.

The following spring, 25 tap samples were taken around the location of each trap, for a total of 100 tap samples per block. Samples were taken as far into the egg hatch period as possible during the time of fruit susceptibility, which ends at petal fall (Reding 2000). Most samples on 'Delicious' were taken at petal fall while most on 'Golden Delicious' were taken the day before the blocks were sprayed for *C. verbasci*, usually between full bloom and petal fall. Each block was determined to be above or below threshold according to the current practice: 1 nymph per tap for 'Golden Delicious' and 4 nymphs per tap for 'Delicious'. Fruit injury was assessed by examining 400 fruits per block *in situ* before harvest.

Apple cultivar susceptibility: Trees of the cultivars ‘Golden Delicious’, ‘Delicious’, ‘Fuji’, ‘Gala’, ‘Granny Smith’, and ‘Cameo’ were selected in orchards in north central Washington in 2002 and 2003. At king bloom, *C. verbasci* nymphs were collected in a heavily infested TFREC orchard near Orondo, WA. Second instars were placed in 15 × 20 cm sleeve cages placed over flower clusters at each site and nymphs were allowed to feed for 1 week. Flowers in the cages were pruned to a single king bloom and two leaves and pollinated before closing the cage. Each cultivar had about 20 cages containing one (2002) or two (2003) *C. verbasci* nymphs and a corresponding number of check (empty) cages. Fruit injury was assessed at petal fall and the number of *C. verbasci* nymphs recorded. A sample of flowers from each cultivar was photographed to compare surface structures, and damaged fruit were photographed at harvest. Data were analyzed using two-way analysis for comparisons of damage/no damage frequency. Data for each cultivar were compared with ‘Golden Delicious’ and ‘Delicious’.

Economic threshold for ‘Gala’: In 2003, six ‘Gala’ trees were selected from a block at the TFREC, Wenatchee, WA, and used for artificial infestation with *C. verbasci* nymphs. Cages consisting of a fabric tube were slipped over a branch of blossom clusters at approximately full bloom. Cages fit over whole sections of branches the width of a beating tray (45 cm), and blossoms were pollinated with crabapple flowers before closing. The treatments were different nymph densities (0, 10, 20, 30, or 40 nymphs/cage), with four replicate cages per treatment. Cages were removed at petal fall, and tap samples for nymphs were taken for each branch. No chemical thinning agents were used; however, trees were hand thinned outside the caged areas. Fruit were counted and evaluated for *C. verbasci* feeding injury at the end of May and after June drop (July). In May, all injury was counted; by July, much of the injury was barely visible and only economically significant damage was counted. A final evaluation was done at harvest in August.

The procedure was repeated in 2004 with the same group of trees. Treatments were 2, 5, 10, and 20 nymphs per cage, with the same number of replicate cages per treatment. At petal fall, tap samples revealed a native nymph population in addition to those added. Fruit were evaluated about a week after petal fall, then before and after June drop, and again at harvest. A linear regression was done for each fruit evaluation period of the proportion fruit damaged and the tap count at petal fall. No intercept was used in the linear regression, forcing the line through the origin.

Results and discussion:

Validation of pheromone trap sampling

Comparison of pheromone trap designs: In the Brewster experiment, the Pherocon 1C caught an average of 26.3 males per trap, whereas the Pherocon IV caught an average of 13.5. The catch of the Pherocon IV was significantly lower (about half) than that of the Pherocon 1C (LSD=8.46, df=3, $\alpha=0.05$). In the second experiment at Orondo, the average catch in the Pherocon 1C trap was 100 males/trap, whereas the average caught in the Pherocon II, Pherocon IIB, and Pherocon IV was 36.0, 83.0, and 54.8, respectively. The catch of the Pherocon II and the Pherocon IV was each significantly lower than that of the Pherocon 1C, whereas the catch of the Pherocon IIB was not significantly different from that of the Pherocon 1C (Dunnett’s test, df=3,9, $\alpha=0.05$).

In both trials, the catch of males in the Pherocon IV was about one-half the catch in the Pherocon 1C. The area of the liner for the Pherocon 1C trap was 63 inches², whereas the area of the liner for the Pherocon IV was 47 inches². Therefore, the area of the sticky trap surface did not explain the difference in catch for the two trap types. Smith (1989) reached the same conclusions about these traps and speculated that the differences were largely the result of the lower “lip” on the delta style trap (same as Pherocon IV) inhibiting entry of flying males. However, this does not invalidate the use of the Pherocon IV, which is easier to handle and maintain. Lowering the threshold for a delta trap by

one-half should detect approximately the same risk as a Pherocon 1C trap. The low risk thresholds for Reding's "long-fall" trapping period, which runs from 1 August to 31 October, is 250 males/trap for 'Golden Delicious' and 350 for 'Delicious' (Reding 2000). Thus, the Pherocon IV thresholds would be 125 for 'Golden Delicious' and 175 for 'Delicious'.

Validation of risk thresholds: The trap technique was designed to indicate nymph density the following spring rather than fruit injury. For 'Delicious', all orchards below the 175 adults/trap threshold in the fall produced nymph densities <4/tap (the current threshold); in fact, most in this

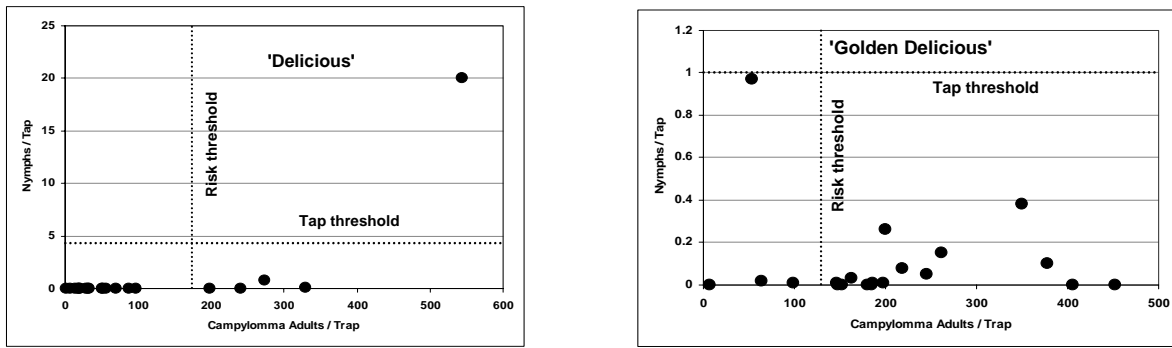


Fig 1. Results of trap catch and tap samples for 10 "Delicious" and 10 "Golden Delicious" orchards over two seasons. The thresholds for "Delicious" are 175 adults/trap and 4 nymphs/tap. Thresholds for "Golden Delicious" are 125 adults/trap and 1 nymph/tap.

group were zeros (Fig. 1).

Four out of 20 cases were false positives, and one case was a true positive (high trap catch resulted in high nymph populations). For 'Golden Delicious', four cases fell below the threshold of 125 adults/trap, and three out of four produced low (near zero) nymph densities. However, there was one false negative, where a trap catch of 54 adults/trap resulted in a near-threshold (1/tap) nymph density for this cultivar. This site had greater than 1% fruit injury at harvest. The remaining cases were false positives. Of these 12 sites, four had fruit injury above 1% at harvest.

The relationship between nymph densities and fruit damage was poor for 'Golden Delicious' (Fig. 2); however, it must be emphasized that all of these orchards were treated for *C. verbasci*. Thus, the resulting fruit damage reflects the efficiency of the control rather than the inherent damage potential

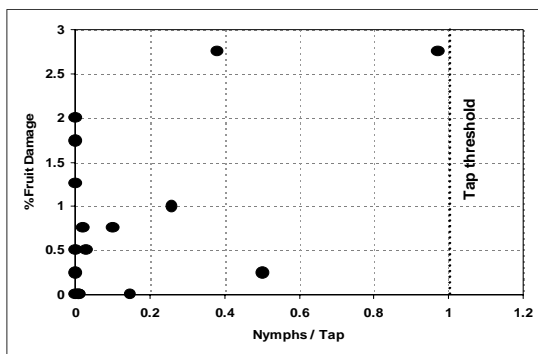


Fig. 2. Nymph samples and fruit damage in 10 "Golden Delicious" orchards over two seasons

on this cultivar. Despite treatment, five of the 16 cases had >1% fruit damage. It appears that the threshold of 1/tap is too high to avoid significant fruit damage (Fig. 2). Indeed, the tap sample threshold (Thistlewood 1986, Thistlewood et al. 1989) for 'Golden Delicious' assumes a higher tolerance of fruit injury. The threshold corresponds to 1% injury for 'Red Delicious' and actually 3% for 'Golden Delicious'.

C. verbasci females may selectively lay eggs on branches with potential prey items for their young, for example, aphids or ERM eggs (Thistlewood 1986). There is speculation (e.g. Edwards 1998) that the lack of prey items during late summer and fall may discourage oviposition in the orchard and thus cause false positive results. However, since many

of the blocks had high populations of secondary pests, especially ERM eggs, this did not explain false positives in any of the orchards. Alternatively, spring applications of chlorpyrifos (which is toxic to *C. verbasci* nymphs) may have caused false positive results in the risk assessments. Reding (2000) proposed higher thresholds for commercial orchards than for untreated orchards. Therefore, the conservative use of thresholds derived from untreated orchards would cause false positive errors in our data.

Apple cultivar susceptibility: In both 2002 and 2003, none of the ‘Braeburn’ fruit in the trial sustained any damage, including the fruit in the check (empty) cages. The percentage of ‘Golden Delicious’ fruit damaged was higher than that of ‘Delicious’ in both years (Fig. 3). These data confirm other research and provisional thresholds that indicate ‘Golden Delicious’ is much more susceptible than ‘Delicious’ (Beers et al. 1993). The percentage of fruit damaged in trials of ‘Gala’, ‘Fuji’, and ‘Granny Smith’ appeared to be intermediate between the percentage damaged in trials of ‘Golden Delicious’ and ‘Delicious’. However, in both years, for these three cultivars, damage was significantly lower than that of ‘Golden Delicious’ but was not significantly different than that of ‘Delicious’ ($2 \times 2 \chi^2$, $\alpha=0.05$). Thus, the economic injury level for ‘Gala’, ‘Fuji’, and ‘Granny Smith’ appears to be somewhat lower than, but close to, that of ‘Delicious’. Based on this assay, a provisional conservative estimate of 2 nymphs/tap could be used for these three cultivars (see next section for refinement of ‘Gala’ threshold). Results for ‘Cameo’ were not consistent between the two years. In 2002, the percentage ‘Cameo’ fruit damaged was significantly lower than that of ‘Golden Delicious’ but was not significantly different from that of ‘Delicious’. In 2003 the results were opposite ($2 \times 2 \chi^2$, $\alpha=0.05$). Pending further investigation, a conservative estimate of 1 nymph/tap could be used for ‘Cameo’.

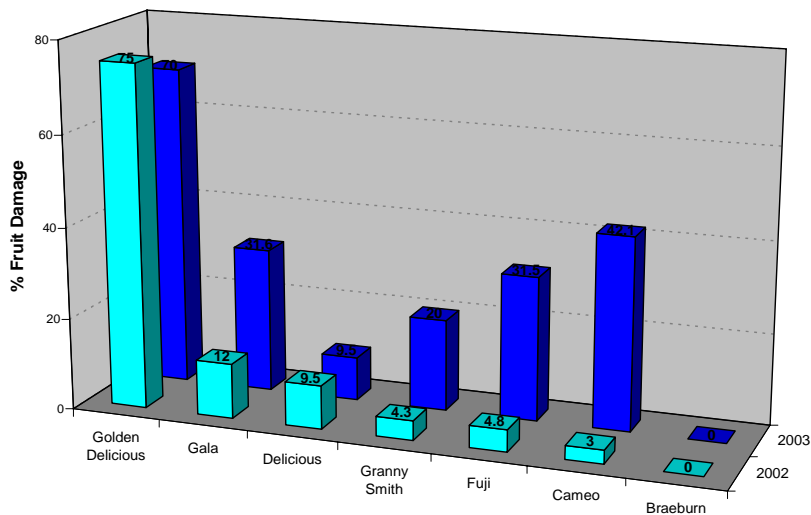


Fig. 3. Percentage of fruit damaged by *C. verbasci* in seven apple cultivars, 2002 and 2003.

Economic threshold for ‘Gala’: Tap samples taken at petal fall, when cages were removed, ranged from 3 to 36 nymphs per tap and recovered an average of 42% of the nymphs introduced in the cages. The branches had an average of 15 flowers along the length of 45 cm. Of these, an average of 9 per branch had formed fruit on 29 May, and 5.8 per branch remained on the tree by 7 July. About the same number, 5.5 per branch, remained on the trees by harvest on 20 Aug. Most of the injuries caused by nymphs were single, raised growths or rough areas smaller than 0.5 mm diameter. By July, many of these were barely visible, less than 1 mm, and resembled an abnormal lenticel (Plates 2a, 2b). A

few injuries were larger than 0.5 mm initially and expanded to a wider area by harvest. Significant injuries sometimes resembled russet (Plates 3a, 3b) or large bumps. Many were surrounded with a dent in the apple surface or caused extreme growth reduction around the feeding site. Some fruit had multiple injuries, resulting in fruit distortion (Plates 4a, 4b). Injury resembling russet has not been described in the literature but may be common in orchards.

	
Plate 2a. Single injury site; not counted as damaged ('Gala', 20 Aug 2003).	Plate 2b. Single bumps, enlarged.
	
Plate 3a. Damage causing fruit skin russet ('Gala', 20 Aug 2003).	Plate 3b. Russeting, enlarged.
	
Plate 4a. Multiple injuries causing fruit distortion ('Gala', 20 Aug 2003).	Plate 4b. Multiple injuries (typical <i>C. verbasci</i> damage) ('Gala', 20 Aug 2003).

After examining the dataset, low numbers of fruit in the formerly caged areas appeared problematic when calculating the proportion of fruit damage, and therefore all replicates with <3 fruits were deleted. In the first evaluation (27 May), the relationship between nymph population and fruit damage was excellent ($F=38.5$, $P=0.0001$, Adj. $R^2=0.73$) (Fig. 4A). At this time, most injury sites were small, and all were counted as damage. The relationship deteriorated by 7 July, when single injury points with no other associated distortion or enlargement were no longer counted as damaged ($F=16.5$, $P=0.001$, Adj. $R^2=0.52$) (Fig. 4B). There was little change from the 7 July to the 20 August evaluation because fruit numbers and damage classification had stabilized; however, the line was slightly flatter by the harvest evaluation ($F=15.1$, $P=0.002$, Adj. $R^2=0.50$) (Fig. 5).

The flattening of the lines over time is an indication of both shedding of damaged fruits and relegating some technically damaged fruits into a non-economic (undamaged) category as the fruit enlarges. The latter phenomenon is shown best by the data points that lie directly on the x-axis (up to 17 average nymphs/tap but no resulting injury) (Fig. 5). Had the fruit in this study been hand-thinned (a normal commercial practice where damaged fruit is selectively removed), the relationship would be even more obscure.

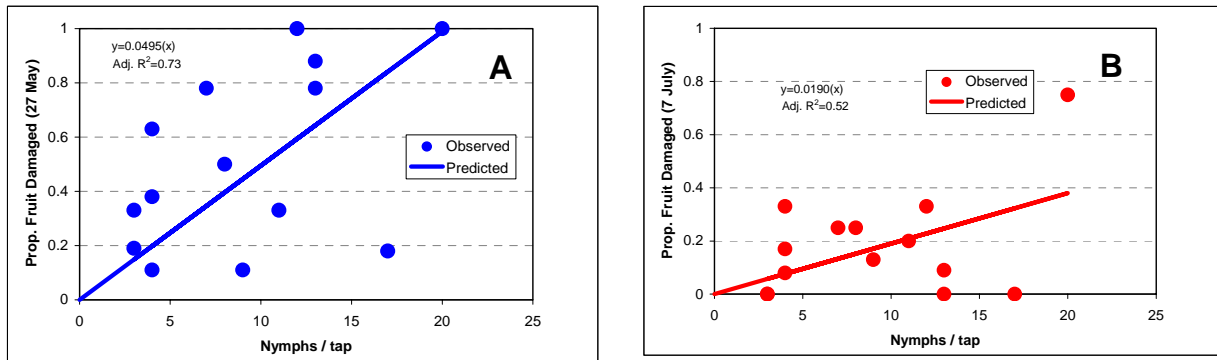
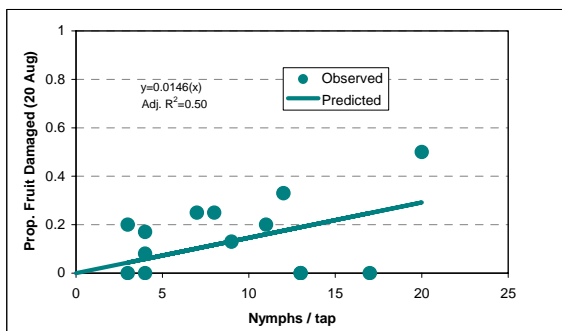


Fig. 4 Regression of nymphs / tap and the proportion of ‘Gala’ fruit damaged on 27 May (A) and 7 July (B) 2003.



In 2004, the first evaluation (3 May) occurred soon after petal fall, before most symptoms developed ($F=18.4$, $P=0.0008$, Adj. $R^2=0.54$) (Fig. 6A). By 8 June, the relationship was strengthened ($F=43.3$, $P=0.0001$, Adj. $R^2=0.74$) (Fig. 6B). When fruit damage could be better classified (6 July) the relationship was less pronounced ($F=26.2$, $P=0.0002$, Adj. $R^2=0.63$) (Fig. 7A), becoming more obscure at harvest ($F=17.8$, $P=0.0009$, Adj. $R^2=0.53$) (Fig. 7B).

Fig. 5. Regression of nymphs/tap and the proportion of ‘Gala’ fruit damaged on 20 Aug 2003.

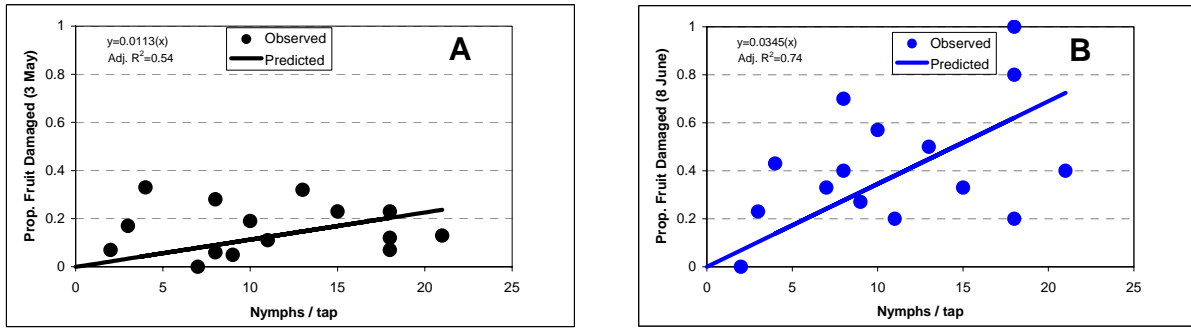


Fig. 6. Regression of nymphs / tap and the proportion of 'Gala' fruit damaged on 3 May (A) and 8 June (B) 2004.

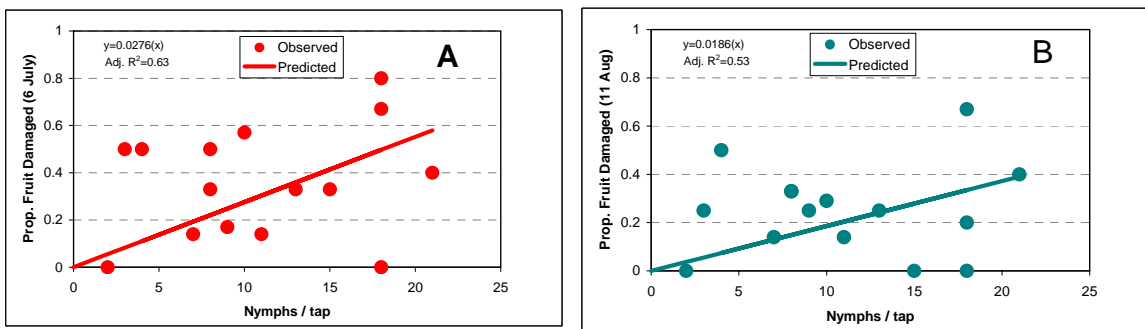


Fig. 7. Regression of nymphs/tap and the proportion of 'Gala' fruit damaged on 6 July (A) and 11 Aug (B) 2004.

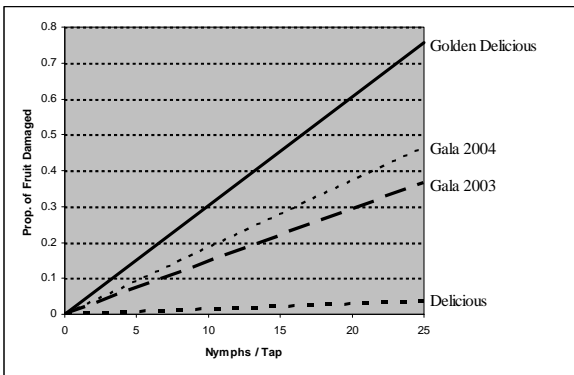


Fig. 8. Regression equations for nymphs / tap and proportion of 'Gala' fruit damaged compared with similar equations for 'Delicious' and 'Golden Delicious' (Thistlewood et al. 1989).

Using the regression developed from the harvest data, an average of 1.5 (2003) and 1.9 (2004) nymphs/tap would produce 1% fruit injury at harvest. A provisional (conservative) threshold of 1 nymph/tap can be used. Although data were collected in different manners, the results can be compared with equations reported for 'Delicious' and 'Golden Delicious' (Thistlewood et al. 1989) (Fig. 8). As in the field assay, 'Gala' appears intermediate in susceptibility to *C. verbasci*.

Microscopic photography of the surface of fruit during flowering revealed great differences in the density of trichomes. 'Golden Delicious', the cultivar most susceptible to *C. verbasci* feeding injury, had the most clearly visible surface (Plate 5a). Other cultivars such as 'Gala' and 'Delicious' had surfaces obscured by dense trichomes (Plates 5b, 5c). The sparse trichomes of 'Golden Delicious' may allow greater access by the mouthparts of *C. verbasci* nymphs and so would allow more frequent feeding. Conversely, cultivars such as 'Gala' and 'Delicious' may be less accessible to nymphs and thus less susceptible to injury.



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Budget:

Project title: Feeding behavior, thresholds, and pheromone trapping of *Campylomma verbasci*
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	Year 1	Year 2	Year 3
Total	\$27,385	\$24,156	\$26,591