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Profile of Fork-Tailed Bush Katydid (Orthoptera: Tettigoniidae) Feeding on Fruit of Clementine Mandarins

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Abstract

Sweet oranges (*Citrus sinensis* (L.) Osbeck Sapindales: Rutaceae) dominated commercial citrus production in California until recently when there has been a shift to mandarins, mostly *Citrus reticulata* (Blanco) mandarins and *Citrus clementina* (hort. ex Tanaka) clementines. Past analyses of commercial field scouting and harvest data indicated that fork-tailed bush katydids (*Scudderia furcata* Brunner von Wattenwyl), a major pest in oranges, are present in clementine groves, but that fruit scarring attributed to katydids is rare. Conversely, jagged or web-like scarring attributed to caterpillars was more prevalent than expected. We used two field experiments in four representative cultivars of clementines to test four explanatory hypotheses for this observation: 1) katydids do not feed on clementine fruit, 2) damaged clementine fruit recover, 3) damaged clementine fruit preferentially abscise, and 4) katydid scars on clementine fruit have a different, undocumented morphology, not recognized as katydid damage. We find support for the latter two hypotheses. Katydids fed readily on the clementine fruit of all cultivars tested, chewing irregular holes that developed into jagged or web-like scars of a range of shapes and often led to splitting and abscission of maturing fruit. The katydid scars often more closely resembled chewing caterpillar damage than the round katydid scars in oranges, suggesting that katydid damage is being misclassified in clementines. The resistance documented in some other mandarins was not observed. Katydids are clearly a frugivorous pest causing previously unrecognized scarring in clementines.

Resumen

La naranja dulce (*Citrus sinensis* (L.) Osbeck Sapindales: Rutaceae) era el producto principal del sector citrícola de California, pero recientemente ha habido un cambio hacia el cultivo de mandarinas, especialmente mandarinas *C. reticulata* (Blanco) y clementinas *C. clementina* (hort. ex Tanaka). Los datos de censos entomológicos y análisis de cosechas indicaban la presencia de katídidos o esperanzas (*Scudderia furcata* Brunner von Wattenwyl Orthoptera: Tettigoniidae) en huertos de clementinas. Los katídidos son una seria plaga de naranjas, pero en los huertos de clementinas se encontraban pocos de los daños que los katídidos causan en frutos. Por otro lado, había más daño por orugas (cicatrices angulares o en forma de red) de lo esperado. Llevamos a cabo dos experimentos de campo usando cuatro variedades de clementina representativas para interrogar cuatro hipótesis que explicaran lo observado: 1) los katídidos no se alimentan de clementinas; 2) las clementinas dañadas se recuperan, 3) la fruta dañada sufre abscisión de manera preferencial, y 4) la cicatrización por katídidos en clementinas presenta una morfología distinta que no ha sido documentada y por ende no es clasificada como daño por katídidos. Nuestros resultados apoyan las dos últimas hipótesis. Los katídidos no demoraron en alimentarse de todas las variedades de clementina que usamos y sus mordeduras dejaban hoyos irregulares que se desarrollaban hasta crear cicatrices angulares o en forma de red con muchos patrones diferentes, las cuales frecuentemente causaban que la fruta se abriera o se perdiera por abscisión. Las cicatrices de katídidos se asemejaban más al daño causado por mordeduras de orugas que a las cicatrices circulares que los katídidos causan en naranjas, lo cual apunta a que el daño que causan los katídidos en las clementinas está siendo clasificado incorrectamente. La resistencia que presentan otras mandarinas

no fue observada en este caso. Claramente los katídidos son una plaga frugívora que causa cicatrización en clementinas que hasta ahora no ha sido reconocida.

Since burgeoning in the late 1800s, fresh market citrus production has been an iconic cornerstone of the Californian economy. Until recently, citrus production was dominated by sweet oranges, mostly navel and Valencia cultivars of *Citrus sinensis*. In the past ca. 20 yr however, there has been a dramatic shift to mandarin production, with cultivars of *C. reticulata* (e.g., 'Tango' and 'W. Murcott Afourer') and *C. clementina* clementines now comprising a substantial share of the newly planted acreage and crop value (CDFA 2018, CDFA and CASS 2018). A number of endemic arthropod pests, such as scales, thrips, katydids, and mites, attack sweet oranges in California and are managed by a year-round integrated pest management (IPM) program established from decades of research and experience in sweet oranges. However, current management guidelines do not adequately represent possible differences in pest status for the increasingly popular mandarin species (Cass et al. 2019a,b; Mueller et al. 2019).

Fork-tailed bush katydids (*Scudderia furcata* Brunner von Wattenwyl Orthoptera: Tettigoniidae) are a key endemic pest in sweet oranges in California. They feed directly on young sweet orange fruit in the spring, chewing deep holes that cause fruit to abscise or leave circular to oval scars that persist to harvest in winter. The scarring leads to downgrading of the fruit quality and loss of value, often motivating aggressive control when katydids are detected in grove scouting during the spring (Grafton-Cardwell et al. 2003, 2017; Dreistadt 2012; Ferguson and Grafton-Cardwell 2014). In contrast to sweet oranges, *C. reticulata* 'Tango' and 'W. Murcott Afourer' mandarin fruit are naturally resistant to katydid feeding. Katydids are present in *C. reticulata* groves but do not generate significant feeding on the mandarin fruit, meaning they are not an economic pest in these fresh market mandarins (Cass et al. 2019a,b). The goal of this study was to assess the status of katydids in *C. clementina*, the other commonly grown mandarin species in California.

A previous analysis of observational scouting and harvest data pooled from commercial citrus farms in Tulare and Fresno counties of California suggested that katydid scarring is lower in *C. clementina* cv. 'Clemenules' clementines (the only *C. clementina* cultivar represented in the data analyzed) than in cultivars of *C. sinensis* sweet oranges, despite similar densities of katydid nymphs in *C. clementina* cv. 'Clemenules' clementine and sweet orange groves (Cass et al. 2019a). Conversely, the jagged or web-like scars thought to be caused by a complex of lepidopteran pests were more common on 'C. clementina cv. 'Clemenules' clementines in comparison to navel oranges, despite similar reported densities of caterpillars on the two citrus species. Here, we conducted two field experiments, one with live katydids and one with mechanically applied, artificial katydid feeding, to profile fruit susceptibility, abscission, and scarring response of four representative cultivars of *C. clementina* clementines. The experiments tested four nonmutually exclusive hypotheses to explain the lower than expected katydid scarring observed in commercial scouting reports: 1) katydids do not feed on *C. clementina* fruit, 2) damaged *C. clementina* recover during development, 3) damaged *C. clementina* fruit preferentially abscise, and 4) katydid scars on *C. clementina* have a novel, undocumented morphology, different from what is observed on sweet oranges.

It is important to test these hypotheses because they have different management implications. If the katydids are present in the groves but the fruit either resist feeding damage (hypothesis 1) or recover from feeding damage (hypothesis 2), the katydids would not need to be managed as significant or important pests in mature groves. If damaged fruit are more likely to abscise (hypothesis 3), the economic outcome of this preferential abscission would depend on the interplay of a number of factors including the timing of the fruit drop and whether the tree can reallocate the resources to other fruit, the effect on the total number of fruit retained, and the market value and labor costs of harvesting damaged fruit. Citrus naturally abort almost all of the fruit set in the 'early drop' during the first few weeks after petal fall and then more still in the 'June drop', with estimates of greater than 99% abscission of the initial fruit set not uncommon (Addicott and Lynch 1955, Erixckson and Brannaman 1960, Zucconi et al. 1978, Stephenson 1981, Iglesias et al. 2007). If the tree can opt to include the damaged fruit in the proportion of fruit that were already going to shed, in favor of reallocating resources to undamaged fruit, this early preferential abscission may have a neutral or positive net impact for growers by removing the damaged fruit without reducing yield. Later in the season in the months prior to harvest, however, additional abscission of damaged fruit is likely to have an overall negative impact as the tree has already invested resources in fruit development. Lastly, if the scars have an undocumented morphology (hypothesis 4), their misclassification could be affecting the management of not just katydids but other pests if the damage is being attributed to the wrong organism.

Materials and Methods

Field Experiment Site

Two field experiments, herein the 'katydid herbivory experiment' and the 'artificial damage experiment', were conducted in 2018 at the University of California Lindcove Research and Extension Center (LREC), which is located in Petal Fall District 1 of Tulare County, California. Petal fall was declared on 24 April 2018, indicating the time at which 75% of the petals had dropped from the trees in the growing district (Tulare County Agricultural Commissioner/Sealer 2018). Petal fall is monitored to lift restrictions on the use of pesticides harmful to bees and was used here as a phenological marker for reporting our experimental dates to help generalize the results to other years and areas. The two experiments were run concurrently in Block 73N, a 0.7-ha plot of 18 different *C. clementina* clementine cultivars on Carrizo rootstock planted in 2004, with the cultivars interspersed and represented in all quadrants of the grove. The four cultivars used in the experiments were 'Clemenules', 'Corsica 1', 'Fina', and 'Fina Sodea', chosen based on their popularity in commercial acreage (CDFA and CASS 2018; and informal grower surveys) and their availability in the field plot. 'Clemenules' or 'Nules' is the most commonly grown clementine in California. 'Clemenules' and 'Corsica 1' are reported to be derived from 'Fina'. 'Fina Sodea' is an early ripening, seedless clementine cultivar (University of California Riverside 2018). The katydid rearing and experimental protocol generally were adapted from Cass et al. (2019b). This grove was not treated with any insecticides during 2018.

Fork-Tailed Bush Katydid Collection and Rearing

Fork-tailed bush katydids aged mostly first and second instar (UCANR Online Learning 2017) were collected between 0800 and 1500 h on 10–11 April from a commercial citrus block of 'Gold Nugget' mandarins and an adjacent commercial blueberry block in Fresno County, California. The groves had not recently been treated with insecticides targeting katydids. The katydids were collected by coaxing them from the plant leaves into 25-ml plastic vials or 1-liter plastic containers, and immediately released into one of four cages (two BugDorm-2400F Insect Tent L75 × W75 × H115 cm, and two BugDorm-2021F Insect Tent L61 × W61 × H61 cm, nylon netting, MegaView Science, Taiwan). The cages were kept in the shade while collecting in the field and then moved indoors and maintained at room temperature. Each cage was provided with two water vials with cotton wicks that were changed approximately weekly when the wicks were soiled or dried out. Each cage was also provided a bouquet of foliage, fruit, and flowers (when flowers were present), from a mix of common citrus species: 'Parent Navel' *C. sinensis* sweet orange, 'Tango' *C. reticulata* mandarin, 'Clausellina' *C. unshiu* Marcovitch satsumas, and 'Clemenules', 'Corsica 1', 'Fina', and 'Fina Sodea' *C. clementina* clementines. The approximately 30-cm cuttings were taken from trees not used in the experiment, from groves that had not been treated with insecticides during 2018. The cuttings were kept in one beaker per cage with water and a floral foam brick (OASIS Micro Brick 3230, Oasis Floral Products), changed approximately every five days when the leaves began to desiccate.

Katydid Herbivory Experiment

Katydids were transferred from the cages individually in 25-ml plastic vials to the field site in a cooler. Each nymph was carefully released into a 19-liter (5 gal) mesh paint strainer bag, enclosing an approximately 30-cm terminal branch length cluster of fruit and leaves. The mesh cages were secured around the branches with wire, and the position of the bag closure was marked with an aluminum identifying tag and flagging tape. Each cluster had between 3 and 20 fruit and a roughly similar amount of foliage. There were no flowers or floral tissue (e.g., styles or petals) present. An equal number of no-katydid control cages was included on each tree. Experimental and control branches were chosen at random within each tree section, interspersed around the full perimeter of the tree, approximately 0.5–2 m from ground level. Treatment cages containing one katydid were alternated with control cages that contained no katydid. Four control and four treatment cages were set up per tree, across five trees per cultivar, for a total of 20 control and 20 treatment replicates per cultivar, for a total of 80 control and 80 treatment replicates. The experimental trees were distributed across the grove. The setup was spread over two days, with the first four trees from each cultivar setup on May 16 and the remaining one tree per cultivar setup on May 17, i.e., days 22 and 23 after petal fall, 2018.

The katydids were confined on the branches for 7 d. After 7 d (i.e., days 29 and 30 after petal fall), the cages were removed and katydid status was noted (alive/dead). Fruit from cages in which the katydid died were excluded, because the length of exposure to the katydid was unknown (6 cages from 'Clemenules', 11 cages from 'Corsica 1', 8 cages from 'Fina', and 9 cages from 'Fina Sodea'). A mean 1.9 ± 2.3 SD (median = 1, range = 0–14) fruit that had abscised into the cage during the confinement interval or between cage removal and tagging were excluded, as many were too desiccated to assess damage or were lost between cage removal and damage assessment. In some katydid treatment cages, all fruits abscised prior to fruit assessment. Each retained fruit was labeled

with a folded tape tag secured loosely on the branch or petiole with a plastic-coated wire twisty-tie. In the remaining 20 control cages per cultivar and 13 'Clemenules', 9 'Corsica 1', 11 'Fina', and 10 'Fina Sodea' katydid treatment cages, there were 6.1 ± 2.9 (range 1–17, total 754) tagged fruit per cage.

Immediately following cage removal and fruit tagging, the tagged fruit were carefully inspected with a hand lens to determine the presence/absence and severity of bite marks. Bite mark severity on the fruit was ranked in levels of increasing severity as: 0, none; 1, individual shallow cut/s, often chevron-shaped or paired (as expected from a bite with paired mandibles, but without fruit tissue obviously removed); 2, extensive surface-level chewing, or many overlapping shallow cuts; or 3, one or more deep, chewed holes or majority of fruit consumed. The proportion of the fruit surface area that was impacted by the feeding was estimated for each fruit. When a fruit had more than one level of damage, only the most severe damage level was recorded. The presence/absence of bite marks on the floral disc (a ring-like pad of tissue between the calyx and the fruit itself), and the proportion of the floral disc that was affected by the bites, were also recorded. This floral disc feeding is shallow relative to the size of the fruit base and unlikely to mechanically cause abscission, but whether it triggers a hormonal abscission response is unknown. It is not likely of economic importance to the grower, but was recorded as it was one of the few plant structures, in addition to the flower petals and stigma, on which the katydids did feed when confined on *C. reticulata* cv. Tango mandarins in similar experiments (Cass et al. 2019b). The measurement of feeding damage to fruitlets tested hypothesis 1 (rejection of clementine fruits as a food source).

Abscission of the tagged fruit was monitored at intervals of every 1–2 mo until harvest on 27 November 2018, 217 d after petal fall, when the fruit were fully developed. This tested hypothesis 3 (elevated abscission of damaged clementine fruit). Fruit scar area was measured and estimated as a rectangle (length × width), and scars were visually assessed for shape and appearance. This tested hypotheses 2 (healing of feeding scars) and hypothesis 4 (unrecognized scar appearance), respectively. When multiple scars were present on a fruit, the areas were added to give a single estimate of scar size per fruit. Fruit weight was recorded as a measure of fruit development.

Artificial Damage Experiment

An artificial damage experiment was conducted concurrently with the katydid herbivory experiment, beginning on 30 May, 36 d after petal fall, 2018. This experiment served two purposes: 1) to independently measure fruit abscission in response to randomly assigned, standardized damage, and 2) to test scar development, determining whether a round hole in young clementine fruitlets results in a typical round scar in mature fruit. Fifteen branches with fruit clusters were selected per tree, on three trees per cultivar, and tagged with aluminum identifying tags and flagging tape. From each branch, three fruit were each labeled with a folded tape tag secured loosely on the branch or petiole with a plastic-coated wire twisty-tie, and assigned to one of three treatments ('heavy damage', 'light damage', and 'no damage' control) for a total of 540 experimental fruit (45 fruit per treatment per cultivar). The experimental trees from each cultivar were from throughout the grove and not the trees used in the katydid herbivory experiment. The 'heavy damage' fruit were punctured with a tubular, 1.5-mm diameter round biopsy punch to a depth of 1.7 mm and the tissue within the biopsied area scratched out with fine forceps, being performed by the same researcher for all fruit to standardize the treatment. The 'light damage' fruit were punctured with a tubular round 0.75-mm diameter biopsy punch to

a depth of 0.5 mm, with the tissue within the punch not removed. The punctures were made at the widest part of the fruit approximately along the fruit equator. At the start of the experiment, the mean diameter of the fruit was 8.1 ± 0.36 mm (range 3–21 mm), as measured with a ruler across the longitudinal axis (calyx to apex) of each fruit as an assessment of fruit development. The tagged fruit were monitored and harvested as per the katydid herbivory experiments to assess abscission and scar development. As the resulting scars were oval to round, the diameter was measured by averaging the length and width of the scar on one harvested fruit per tree in the 'heavy damage' and 'light damage' treatments. The weight of one harvested fruit per tree in each treatment category was measured as an assessment of fruit development.

Statistical Analyses

Statistical analyses and graphing were performed in R version 3.6.1 (R Core Team 2018) using R packages in *tidyverse* (Wickham et al. 2019). For the katydid herbivory experiments, each fruit was considered a separate observation with *fruit damage level* as an ordinal response variable. The effect of the predictor variable *katydid exposure* on the ordinal response variable *fruit damage level* was tested using one-way repeated ordinal regression (cumulative link mixed model, CLMM) with *cage identifier* nested in *tree identifier* as a random effect, with threshold 'flexible', using the *clmm* function in the R package *ordinal* (Christensen 2019). The assumption of proportional odds for the model was verified on a *clm* version of the model without random effects using the *nominal_test* and *scale_test* functions. The effect of the predictor variable *katydid exposure* on the response variables *feeding damage to floral discs* was tested using a generalized linear mixed effect model (GLMM) with a binomial family distribution (number damaged, number undamaged per cage), *cultivar* as a covariate, and *tree identifier* as a random effect using the *glmer* function in the R package *lme4* (Bates et al. 2015). Type II analysis of variance was performed for categorical predictors using the R packages *car* (Fox and Weisberg 2019) and *RVAideMemoire* (Hervé 2020). When there was a significant effect of *cultivar*, paired contrasts were performed with Tukey adjustment using the R package *emmeans* (Lenth 2020).

To assess fruit abscission rates in the katydid herbivory and artificial damage experiments, the effect of the predictor variable *fruit damage level* on the response variable *fruit abscission time* (day from tagging to day on which the fruit was first recorded as abscised) was tested using Cox mixed effects survival models (Cox), in the R package *coxme* version 2.2–14 (Therneau 2019) for the one-tailed test of the hypothesis of increased fruit abscission with damage. In the katydid herbivory experiments, *cage identifier* nested in *tree identifier*, and in the artificial damage experiments, *tree identifier* were included as random effects. The predictor *feeding damage to floral discs* was not included due to collinearity with the fruit damage predictor. Fruit that survived to harvest were right-censored. In the katydid herbivory experiments, there was some minimal background damage in the controls that probably occurred before the cages were applied, and not all fruits within the bags were damaged by katydid feeding. As we wanted to test for preferential abscission in response to feeding damage, not just in response to katydid exposure (even for fruits that were not bitten), fruit abscission was assessed based on the initial damage assessment score category, regardless of exposure to katydids, meaning that a few fruits with damage were from no-katydid control cages, and many fruits with no damage had been exposed to katydids. Due to the high natural abscission levels in citrus causing the majority of experimental fruit to abscise in the

first few weeks, and as cultivar did not significantly affect abscission (as reported in Results), the models were run with data from all cultivars pooled, with the factor *cultivar* included as a predictor, rather than run separately for each cultivar. For the katydid herbivory experiment, to determine whether the main differences in abscission were in the early period of high natural fruitlet abortion or later in the preharvest drop period, survival analyses on subsets of the data censored at 65 d post-petal fall (early abscission period) and for fruit that were still present at 65 d post-petal fall (late abscission period) were run. We pooled the harvested fruit to describe scar size trends and weight, as there were few retained fruit and no clear differences in trends among the cultivars.

Results

Katydid Herbivory Experiment

Katydids fed on many of the clementine fruitlets, generating a range of damage (examples of feeding damage shown in Fig. 1a–h). The damage ranged from small, individual, shallow cuts (damage level 1, Fig. 1a and b) that covered an estimated mean 5.6 ± 10.1% (range 1–70%) of the fruit surface; to multiple overlapping bites creating contiguous areas of superficial damage (damage level 2, Fig. 1c) that covered an estimated mean 16.0 ± 15.4% (range 10–70%) of the fruit surface; to deep holes (damage level 3, Fig. 1d–h) that covered an estimated mean 34.8 ± 22.2% (range 5–100%) of the fruit surface. In some cases, the fruit were chewed off from the base (Fig. 1g) or completely consumed down to the floral disc (Fig. 1h). Katydids fed extensively on the fruit of all cultivars examined; katydid exposure, but not citrus cultivar, was a significant predictor of fruit damage (CLMM, *katydid exposure* χ^2 = 138.7, df = 1, P < 0.0001; *cultivar* χ^2 = 1.1, df = 3, P = 0.770). The background damage levels in the control cages (not exposed to katydids) was low (~10% of fruit with some damage) and almost all present as individual small scratches likely from branch rubbing present before the experiment was initiated, whereas the majority (>80%) of fruit exposed to katydids had some damage, with ~30% having deep chewed holes (Fig. 2a). Katydids also fed on the floral discs (examples indicated with arrows in Fig. 1a and d). In katydid treatment cages, >25% of floral discs were damaged compared to <5% of discs in control cages (Fig. 2b). Floral disc feeding occurred in all cultivars examined, with katydid exposure and citrus cultivar significant predictors of floral disc damage (GLMM, *katydid exposure* χ^2 = 80.7, df = 1, P < 0.0001; *cultivar* χ^2 = 9.0, df = 3, P = 0.029). Floral disc damage was slightly more common overall in 'Fina Sodea' than 'Clemenules' (paired contrasts with Tukey adjustment, Z = 2.845, P = 0.023). The bites to the floral discs covered an estimated mean 3.0 ± 3.0% (range 1–20%) of the floral disc area.

As the fruit matured, the damaged fruit were more likely to abscise than undamaged fruit, with abscission of heavily damaged fruit occurring throughout the season, including late in the season up to harvest (Fig. 3). This was the case for all cultivars, with initial damage level but not citrus cultivar a significant predictor of abscission (Cox, *initial damage* χ^2 = 7.1, df = 3, P = 0.034; *cultivar* χ^2 = 2.2, df = 3, P = 0.266). The effect of damage on fruit abscission was significant in the late abscission period (Cox, χ^2 = 14.6, df = 3, P = 0.001) not the early (Cox, χ^2 = 3.5, df = 3, P = 0.162). Late abscission of fruits, occurring between days 65 and 217 after petal fall, was minimal in fruits with either no damage (damage level 0, 11.8% abscission) or fruits with small cuts (damage level 1, 6.1% abscission) or surface chewing (damage level 2, 5.3% abscission), but was substantial on fruits
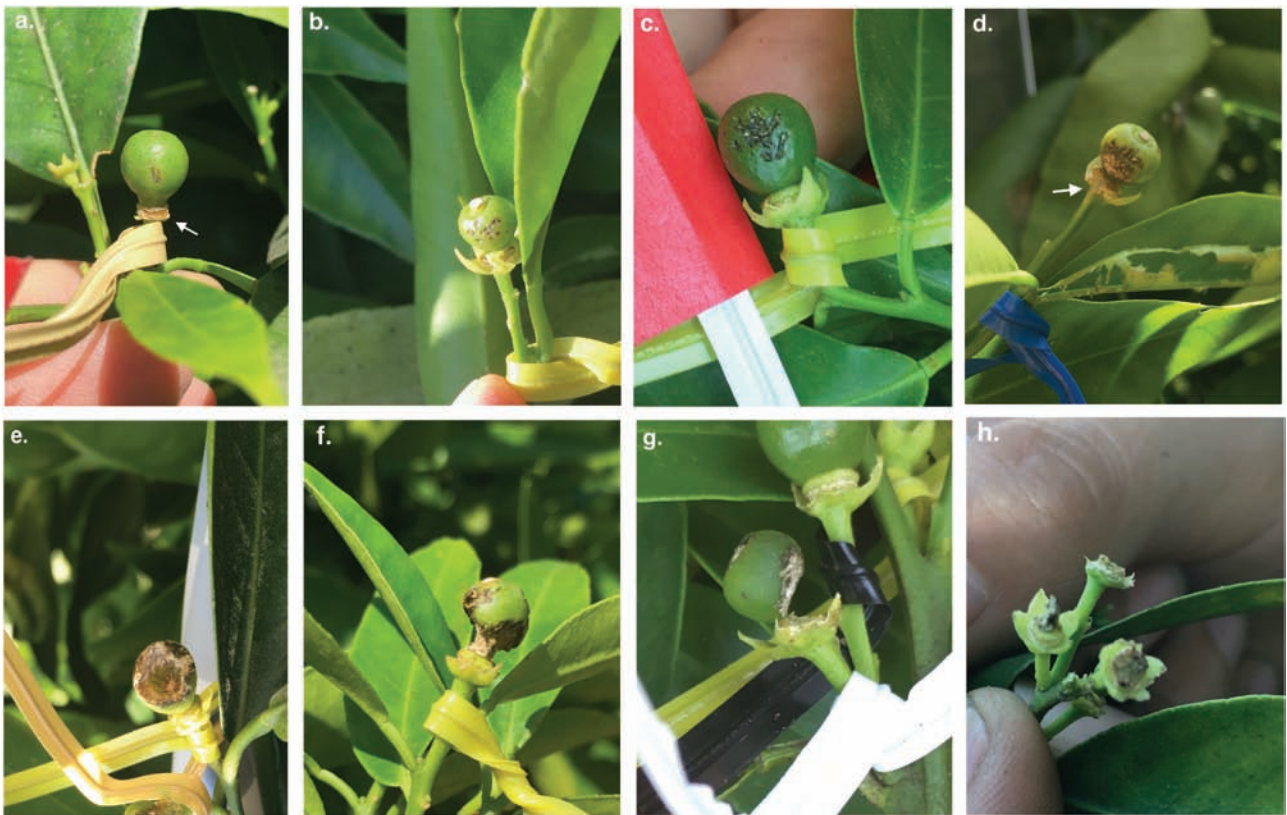
Fig. 1. Examples of katydid feeding damage to young *Citrus clementina* clementines. Damage included small, individual, shallow cuts (a and b), multiple overlapping bites creating contiguous areas of superficial damage (c), and deep holes (d–h) that in some cases dislodged (g) or completely removed the fruit (h). Feeding to the floral disc can be seen in photos (a) and (d), marked with white arrows.
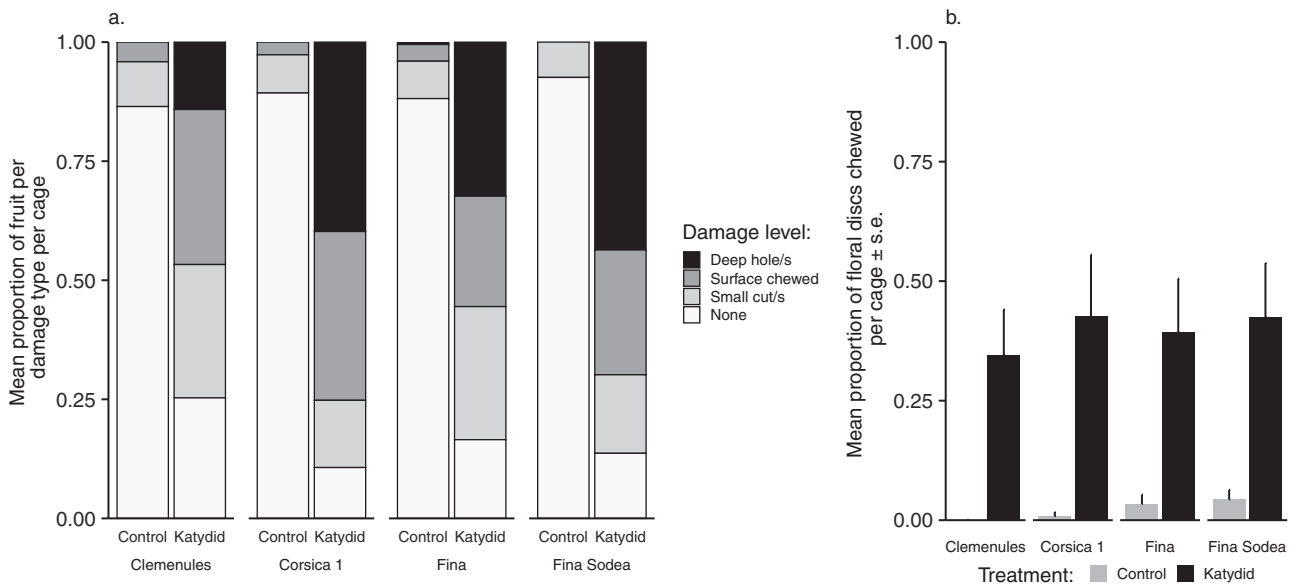

Fig. 2. In the katydid herbivory experiment: katydids generated substantial feeding damage on (a) young fruit, and on (b) floral discs of all cultivars of *Citrus clementina* clementines tested. The damage in the control cages was likely mechanical damage or preexisting feeding damage from before the treatments were applied.

with deep holes (damage level 3, 62.5% abscission). The maturing fruit developed substantial scarring where the katydids had fed, in many cases having long, irregular, or jagged scars (Fig. 4a). At the monitoring timepoint in October, we noticed that several of

these nearly mature fruit had split along the katydid scar and subsequently abscised (Fig. 4b).

At harvest, the retained fruit from katydid treatment cages had a range of scar morphologies (examples in Fig. 4c–i). In some cases
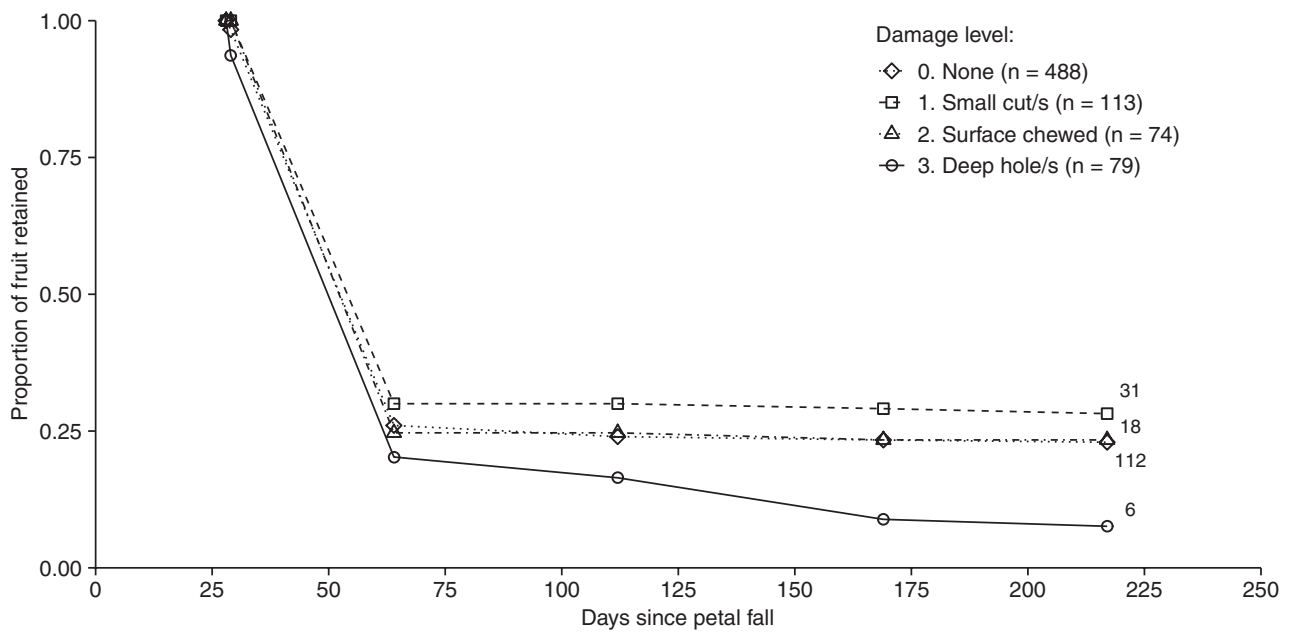
Fig. 3. Katydid damage significantly increased fruit abscission, with abscission of the heavily damaged fruit continuing late into the season as the fruit matured to harvest. Numbers at the ends of the censored retention curves are the number of fruit remaining at harvest.
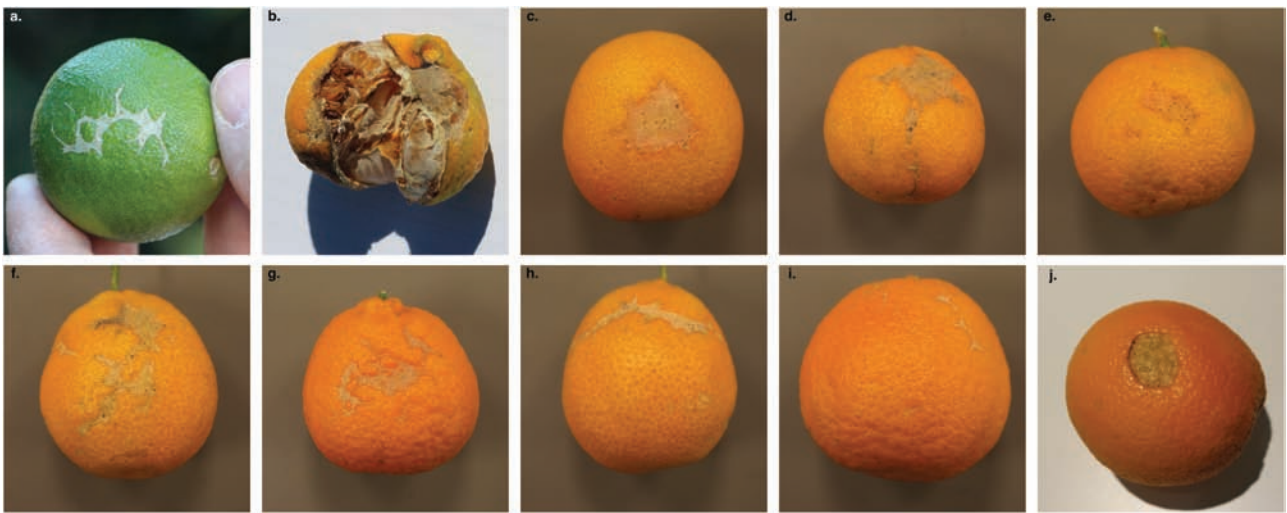

Fig. 4. Examples of scarring on *Citrus clementina* clementines that had been fed on by katydids early in the season. Some maturing fruit developed irregular, jagged scars (a) similar to damage attributed to caterpillars in sweet oranges. At the monitoring timepoint in October, we noticed that several of these nearly-mature fruit had split along the katydid scars and subsequently abscised (b). At harvest, the retained fruit from katydid treatment cages had a range of scar morphologies including: large, deep, roundish and scabby (c and d); smaller, deep, and scabby (e); deep, scabby and irregular/webbed (f and g); thin and jagged with other small marks (h and i). An example of the circular scarring typical of katydid damage in sweet oranges is provided in photo (j) for comparison.

(Fig. 4c and d), the scars were large, deep, and scabby, somewhat similar to the circular scars typical of katydid damage in sweet oranges (example provided in Fig. 4j for comparison) but less round. Other scars were deep and scabby but smaller (Fig. 4e), or deep, scabby, and irregular/webbed (Fig. 4f–h). Some scars were thin or small (Fig. 4i). Six fruit from the katydid treatment cages that initially had heavy feeding damage (deep chewed holes) were retained to harvest, and all had large scars that covered an average fruit surface area of 7.0 ± 5.8 cm² (range 0.7–16.7 cm²). Fourteen fruit from katydid treatment cages that initially had an area of the surface chewed were retained to harvest, and 11 of them had scars, but these

scars were smaller, covering an average area of 2.0 ± 4.2 cm² (range 0.1–14.4 cm²). Thirteen fruit from katydid treatment cages that initially had individual, shallow bite marks were retained to harvest, and 10 of them had small scars at harvest covering an average area of 1.5 ± 1.5 cm² (range 0.1–3.9 cm²). Some background damage was also present at harvest; one out of the eight katydid-exposed fruit as well as 20 of the 126 harvested fruit from control cages that were initially undamaged had some scarring at harvest. These 'background damage' scars, which may have been due to wind damage or to feeding damage by nonexperimental pests present in the grove that occurred after the cages were removed, had an average area of
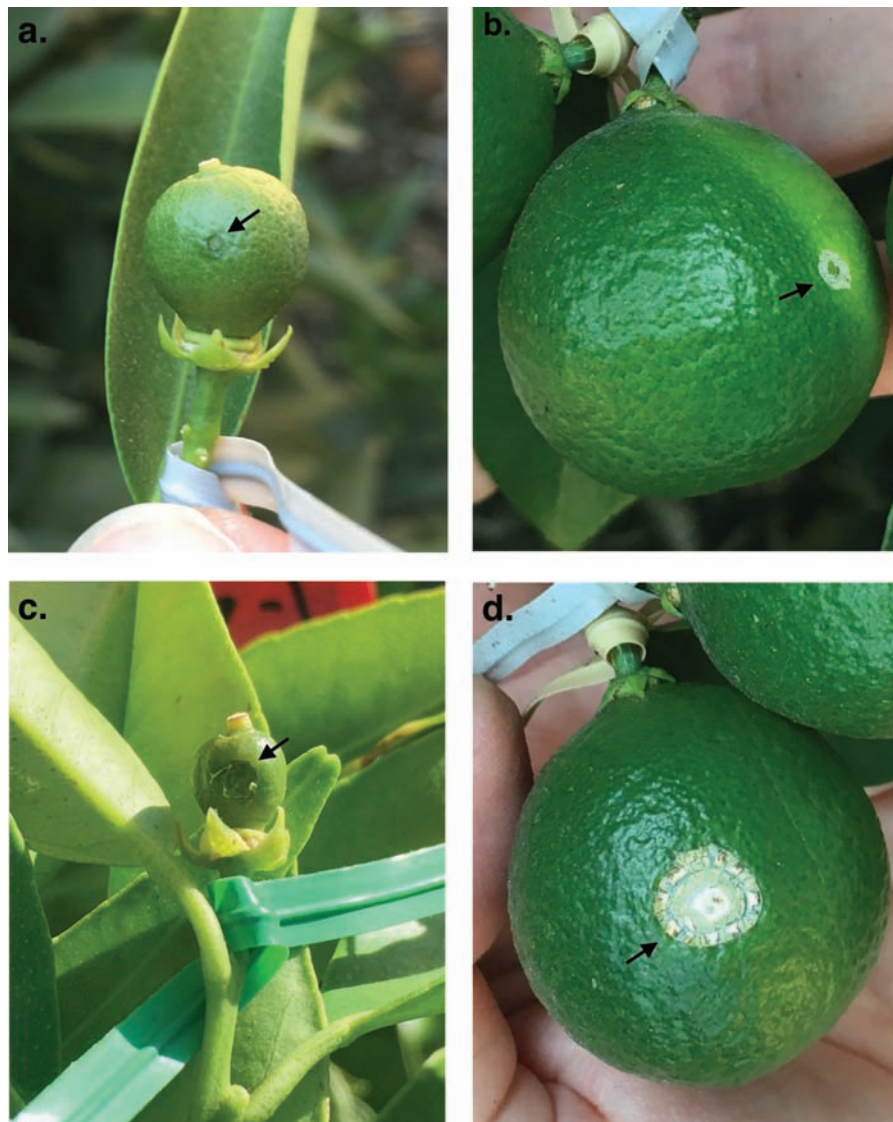
Fig. 5. Examples of the 'light' version of the mechanically applied, artificial damage (a), which resulted in circular scars on the maturing fruit (b); and the 'heavy' version of the mechanically applied, artificial damage (c), which resulted in circular scars on the maturing fruit (d). Damage and scarring indicated with black arrows.

0.4 ± 0.4 cm^2 (range 0.1–1.2 cm^2). The mean weight of the harvested fruit was 68.8 ± 22.5 g (range 22.7–154.6 g).

Artificial Damage Experiment

The 'light' version of the mechanically applied, artificial damage (Fig. 5a) resulted in circular scars on the maturing fruit (Fig. 5b). These small scars had a mean diameter of 3.1 mm $\pm$ 1.4 SD (range 1–7 mm) at harvest. The 'heavy' version of the mechanically applied, artificial damage (Fig. 5c) resulted in circular scars on the maturing fruit (Fig. 5d). These larger scars had a mean diameter of 9.8 ± 3.1 mm (range 6–18 mm) at harvest. The mean weight of the harvested fruit was 79.6 ± 27.4 g (range 36.2–156.8 g).

There was no significant effect of the mechanically applied, artificial damage on fruit abscission (Fig. 6), with abscission rate similar among fruit with no damage, light, or heavy damage, and from all cultivars tested (Cox, *damage treatment* $\chi^2 = 4.1$, df = 2, $P = 0.065$; *cultivar* $\chi^2 = 5.7$, df = 3, $P = 0.064$). There was no effect of simulated feeding damage on fruit abscission for models run separately for each cultivar (data not shown).

Discussion

We found strong support for hypotheses 3 (elevated abscission of damaged clementine fruit) and 4 (unrecognized scar appearance on clementines). We did not find support for hypotheses 1 (rejection of clementine fruit as a food source) or 2 (healing of feeding scars). Our experiments have demonstrated that katydids can generate substantial direct fruit-feeding damage in four cultivars of *C. clementina* clementine mandarins now commonly grown in California. The katydid nymphs fed heavily on the young fruit. In some cases, they consumed the entire fruit or chewed into the stem or fruit base enough to immediately cause the abscission of the fruit. The damaged fruit did not recover, and instead developed scars that persisted to harvest. The scars were often long and irregular with jagged edges, and there were often multiple scars per fruit. The fruit injured with the round biopsy punch did develop round scars, indicating that the jagged scars resulting from katydid feeding were not due to the fruit maturation converting round holes into jagged scars. Rather, it suggests that the irregular scar shape resulted in some other way, for example, from a more diffuse feeding pattern or interaction between the fruit
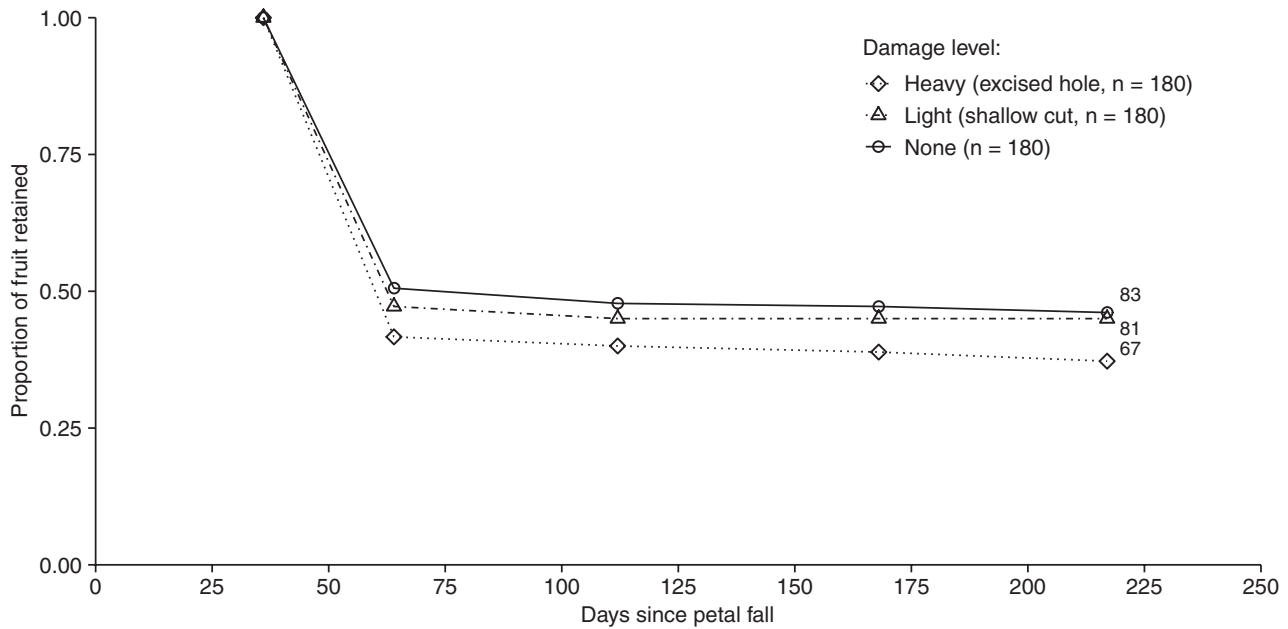
Fig. 6. In the artificial damage experiment, mechanical damage to the fruitlets did not significantly influence fruit abscission. The numbers at the end of the retention curves are the number of fruit remaining in each treatment at harvest.

tissue and katydid feeding. Katydid feeding damage increased fruit abscission late in the season in the preharvest drop period, and in some cases with fruit splitting along the scar.

These results combined with results of our earlier studies (Cass et al. 2019a,b) suggest that there are three very different profiles of katydids in the three commercially grown citrus species considered here. In *C. sinensis* sweet oranges, katydids feed on young fruit, causing distinctive round, regular scars in the retained fruit. In *C. reticulata* 'Tango' and 'Afourer' mandarins, katydid nymphs can be found in the groves but do not feed heavily on the fruit or cause substantial fruit scarring. In *C. clementina* clementines, katydids feed on young fruit, causing irregular-shaped scars that are associated with higher abscission in the preharvest period, and often similar in appearance to scarring produced by chewing caterpillars (Grafton-Cardwell et al. 2003). This provides a possible explanation for the perplexing observations from PCA-generated data that clementines had very low katydid scarring but high caterpillar damage compared with navel oranges, despite similar densities of the katydids in the weeks after petal fall (Cass et al. 2019a). This suggests that katydid scarring is being misclassified as caterpillar damage, leading to an underestimation of katydid scarring and an overestimation of caterpillar scarring in commercial clementine harvests.

The effect of the increase of preharvest fruit drop is somewhat difficult to assess. As the tree has already invested resources maturing the damaged fruit by 65 d post-petal fall, there is more likely to be a reduction in yield. However, abscission saves the farmer the cost of harvesting fruit destined to be downgraded to 'juice' quality at the packing house, where it is of little value in the fresh fruit market for California citrus. We did not observe this late abscission in the artificial damage experiment, but the simulated feeding method we used was modeled on the anticipated damage morphology from observations in oranges. The higher abscission observed in response to katydid herbivory was likely in part due to the fruit splitting along large, long scars, which were not produced by the biopsy-punch circular damage. We did not specifically test whether the splitting was a result of katydid feeding, but for

the several fruit that we observed to have split, it was invariably along a scar line. Some clementine cultivars including 'Clemenules' and 'Fina' are known to be prone to splitting (Cronjé et al. 2013). There are grower recommendations available to reduce the incidence of spontaneous splitting and late fruit drop from other causes (Anthony et al. 1999, Aliviela et al. 1994, Mesejo 2016), but it is unknown whether these measures would help with splitting and fruit drop in groves that have been attacked by katydids, and efforts to reduce the splitting would only serve to retain scarred fruit that are costly to growers.

We did not find strong evidence for an effect of preferential early abscission of damaged fruit. However, in both experiments and in previous work (Cass et al. 2019b), there was a trend toward increased abscission of the heavily damaged fruit, which may mean there is a weak effect that would be more noticeable at the scale of a commercial grove and across the full season including the first few weeks after petal fall before our experiments were initiated. Fruit drop is known to be nonrandom in citrus; for example, smaller fruitlets are preferentially abscised (Agustí et al. 1982). Increased fruit abscission in response to arthropod feeding damage has been reported in other citrus species. For instance, damage by thrips species (Childers and Bullock 1999, Planes et al. 2014) and citrus rust mite (Allen 1978, Yang et al. 1994) has been associated with increased abscission. If the tree is able to shed damaged fruit early by including them in the total proportion of fruit destined to abscise, this would effectively remove damaged fruit from the citrus grove without any corresponding reduction in yield. If the tree increases the overall early abscission rate to remove the damaged fruit, the number of mature fruit may be lower, but they may be larger, as the tree can divert extra resources to the remaining fruit. The economic outcome may depend on the price premiums associated with fruit sizes; if larger fruit are more valuable, it may offset the reduction in yield and cost of control measures. For example, this is the case when citrus red mites infest navel oranges in the San Joaquin Valley, trees produce fewer, but larger fruit (Hare et al. 1990), but larger clementines, may not be of higher value. Future work assessing effects on yield would

help to delineate the potential outcomes of early and late preferential abscission in response to katydid feeding.

This analysis highlights the utility of complementing observational ecoinformatics studies with experimental work. For *C. reticulata* mandarins, we had found support for the first of four hypotheses that were tested as explanations for reduced katydid scarring on fruit in commercial citrus (katydids do not feed on the fruit), whereas here we found evidence for a combination of the third and fourth hypotheses: damaged *C. clementina* fruit preferentially abscise, and katydid scars on *C. clementina* have a novel and previously undocumented morphology that is similar to scarring associated with damage from caterpillar feeding. Ongoing work is testing whether the high katydid mortality observed in this experiment and in experiments in *C. reticulata* (Cass 2019b) is due to these citrus species being a poor food source overall for katydids. Some mortality may also have been due to the confinement of katydids on the branches or the unknown history of the katydids prior to collection.

Our results suggest a need to monitor for, and control, katydids on clementines. Furthermore, the interpretation of damage found at harvest in bin samples for clementines needs to be updated, as the irregular scars could be either caterpillar or katydid scars. Control methods for caterpillars and katydids are quite different, making it important to distinguish between them. Caterpillars such as citrus cutworm have effective natural enemies and selective *Bt*- and methoxyfenozide-based insecticides available. Katydids do not have effective natural enemies and are often treated with broad-spectrum pyrethroids or organophosphates for chemical control, which are fast acting to prevent fruit damage. Selective kryocide or diflubenzuron chemical options for katydids are slower acting so are generally used when katydids are detected before petal fall (Grafton-Cardwell et al. 2017). Overall, we are furthering our understanding of the interactions between the endemic arthropods, such as katydids (here and Cass et al. 2019a,b) and citrus thrips (Mueller et al. 2019), and the major groups of mandarins that have been expanding in acreage in California.

Acknowledgments

Conflicts of interest

The authors declare no conflicts of interest.

References Cited

Addicott, F. T., and R. S. Lynch. 1955. Physiology of abscission. Annu. Rev. Plant Physiol. 6: 211–238.

Agustí, M., F. García-Marí, and J. L. Guardiola. 1982. The influence of flowering intensity on the shedding of reproductive structures in sweet orange. Sci. Hortic. 17: 343–352.

Aliviela, V., S. Zaragoza, E. Primo-Millo, and M. Agusti. 1994. Hormonal control of splitting in 'Nova' mandarin fruit. J. Hortic. Sci. 69: 969–973.

Allen, J. C. 1978. The effect of citrus rust mite damage on citrus fruit drop. J. Econ. Entomol. 71: 746–750.

Anthony, M. F., and Coggins Jr, C. W. 1999. The efficacy of five forms of 2,4-D in controlling preharvest fruit drop in citrus. Sci. Hortic. 81: 267–277.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67: 1–48.

Cass, B. N., E. E. Grafton-Cardwell, and J. A. Rosenheim. 2019a. Resistance of fruits from a mandarin cultivar to feeding by fork-tailed bush katydids. J. Econ. Entomol. 112: 2861–2871.

Cass, B. N., L. M. Hack, E. E. Grafton-Cardwell, and J. A. Rosenheim. 2019b. Impacts of fruit-feeding arthropod pests on oranges and mandarins in California. J. Econ. Entomol. 112: 2268–2277.

(CDFA) California Department of Food and Agriculture. 2018. California agricultural statistics review, 2017–2018. California Department of Food and Agriculture, Sacramento, CA.

(CDFA) California Department of Food and Agriculture and (CASS) California Agricultural Statistical Service. 2018. 2018 California citrus acreage report. USDA's National Agricultural Statistics Service (NASS), Sacramento, CA.

Childers, C. C., and R. C. Bullock. 1999. Controlling *Frankliniella bispinosa* (Thysanoptera: Thripidae) on Florida citrus during bloom and increased fruit set on navel and 'Valencia' oranges. Fla. Entomol. 82: 410–424.

Christensen, R. H. B. 2019. Ordinal – regression models for ordinal data. R package version 2019.12-10. URL https://CRAN.R-project.org/package=ordinal (accessed 17 September 2020).

Cronjé, P. J., O. P. Stander, and K. I. Theron. 2013. Fruit splitting in citrus. Hortic. Rev. 41: 177–200.

Dreistadt, S. H. 2012. Integrated pest management for citrus, 3rd ed. University of California Agriculture and Natural Resources (UC ANR) Statewide Integrated Pest Management Program Publication 3303, Oakland, CA.

Erixckson, L. and B. L. Brannaman. 1960. Abscission of reproductive structures and leaves of orange trees. Proc. Am. Soc. Hortic. Sci. 75: 222–229.

Ferguson, L., and E. E. Grafton-Cardwell. 2014. Citrus production manual. University of California Agriculture and Natural Resources (UC ANR) Publication 3539, Oakland, CA.

Fox, J., and S. Weisberg. 2019. An {R} companion to applied regression, 3rd ed. Sage, Thousand Oaks, CA.

Grafton-Cardwell, E. E., N. V. O'Connell, C. E. Kallsen, and J. G. Morse. 2003. Photographic guide to citrus fruit scarring. University of California Agriculture and Natural Resources (UC ANR) Publication 8090, Oakland, CA.

Grafton-Cardwell, E. E., B. A. Faber, D. R. Haviland, C. E. Kallsen, J. G. Morse, N. V. O'Connell, P. A. Phillips, J. E. Adaskaveg, J. O. Becker, C. J. Lovatt, et al. 2017. UC IPM citrus pest management guidelines for agriculture. University of California Agriculture and Natural Resources (UC ANR) Publication 3441, Oakland, CA.

Hare, D. J., J. E. Pehrson, T. Clemens, J. L. Menge, C. W. Coggins Jr., T. W. Embleton, and J. L. Meyer. 1990. Effects of managing citrus red mite (Acari: Tetranychidae) and cultural practices on total yield, fruit size, and crop value of 'Navel' orange. J. Econ. Entomol. 83: 976–984.

Hervé, M. 2020. RVAideMemoire: Testing and Plotting Procedures for Biostatistics. R package version 0.9–74. URL https://CRAN.R-project.org/package=RVAideMemoire (accessed 17 September 2020).

Iglesias, D. J., M. Cercós, J. M. Colmenero-Flores, M. A. Naranjo, G. Ríos, E. Carrera, O. Ruiz-Rivero, I. Lliso, R. Morillon, F. R. Tadeo, et al. 2007. Physiology of citrus fruiting. Braz. J. Plant Physiol. 19: 333–362.

Lenth, R. 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.7. URL https://CRAN.R-project.org/package=emmeans (accessed 17 September 2020).

Mesejo, C., C. Reig, A. Martínez-Fuentes, G. Gambetta, A. Gravina, and M. Agustí. 2016. Tree water status influences fruit splitting in Citrus. Sci. Hortic. 209: 96–104.

Mueller, T. G., H. M. Kahl, B. N. Cass, E. E. Grafton-Cardwell, and J. A. Rosenheim. 2019. Differential impacts of citrus thrips across sweet orange and mandarin species. J. Econ. Entomol. 112: 2767–2773.

Planes, L., J. Catalan, A. Urbaneja, and A. Tena. 2014. Within-tree and temporal distribution of *Pezothrips kellyanus* (Thysanoptera: Thripidae) nymphs in citrus canopies and their influence on premature fruit abscission. Environ. Entomol. 43: 689–695.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annu. Rev. Ecol. Syst. 12: 253–279.

Therneau, T. M. 2019. coxme: mixed effects cox models. R package version 2.2–10. URL https://CRAN.R-project.org/package=coxme (accessed 17 September 2020).

Tulare County Agricultural Commissioner/Sealer. 2018. Petal Fall Declared in District 1. News Release April 20, 2018. County of Tulare, California.

(UCANR) University of California Agriculture and Natural Resources Online Learning. 2017. Citrus IPM: Forktailed bush katydid, Integrated Pest Management. URL http://class.ucanr.edu/ (accessed 9 April 2020).

University of California Riverside. 2018. University of California Riverside Citrus Variety Collection. URL https://citrusvariety.ucr.edu/ (accessed 21 February 2020).

Wickham, H., M. Averick, J. Bryan, W. Chang, L. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, et al. 2019. Welcome to the Tidyverse. J. Open Source Softw. 4: 1686.

Yang, Y., J. C. Allen, J. L. Knapp, and P. A. Stansly. 1994. Citrus rust mite (Acari: Eriophyidae) damage effects on 'Hamlin' orange fruit growth and drop, Environ. Entomol. 23: 244–247.

Zucconi, F., S. P. Monselise, and R. Goren. 1978. Growth abscission relationships in developing orange fruit. Sci. Hortic. 9: 137–146.