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Resistance of Fruits From a Mandarin Cultivar to Feeding by Fork-Tailed Bush Katydids

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Abstract

Plants use a variety of mechanisms to defend against herbivore damage, each with different consequences for agricultural production. Crops relying on tolerance strategies may need different pest management approaches versus those relying on resistance strategies. Previous work suggested that densities of fork-tailed bush katydids (*Scudderia furcata* Brunner von Wattenwyl [Orthoptera: Tettigoniidae]) that generated substantial scarring on cultivars of sweet oranges (*Citrus sinensis*, (L.) Osbeck [Sapindales: Rutaceae]) produced only low levels of scarring on cultivars of *Citrus reticulata* Blanco mandarins. We used field experiments in representative cultivars of these species to test non-mutually exclusive hypotheses regarding the mechanisms underlying this observation: 1) katydids are averse to feeding on mandarin fruits, 2) damaged mandarin fruits preferentially abscise, 3) damaged mandarin fruit tissue recovers during development, and 4) katydid scars on mandarins have a different morphology that may result in misclassification. We found strong support for the first hypothesis, demonstrating that katydids reject opportunities to feed on *C. reticulata* fruit. Instead of chewing deep holes in the fruit, as was commonly observed for *C. sinensis*, the katydids only scratched the surface of the *C. reticulata* fruits. The hypotheses of preferential abscission of damaged fruits and of recovery of damaged tissue were not supported. The low incidence of damage to the mandarins prevented a comprehensive assessment of the scar morphology; however, at harvest, the superficial cuts in *C. reticulata* were not easily distinguishable from background damage. This indicates that in contrast to *C. sinensis*, *C. reticulata* has substantial natural resistance to fork-tailed bush katydids making them a non-pest in this crop.

Key words: abscission, cultivar effect, citrus species, ecoinformatics, herbivory

There is increasing interest in improving agricultural sustainability by employing natural plant defense traits to reduce pest pressure, rather than relying on pesticides (War et al. 2012, Mitchell et al. 2016). Plants are locked in an evolutionary arms race with herbivores and use a variety of resistance and tolerance mechanisms to reduce damage and moderate the fitness reduction imposed by herbivory (Fineblum and Rausher 1995, Núñez-Farfán et al. 1996, Strauss and Agrawal 1999, Hanley et al. 2007). Resistance strategies include physical/structural defenses and chemical defenses to prevent or reduce damage (Ehrlich and Raven 1964, Levin 1973, Berenbaum et al. 1986, Wink 2003). For example, in sour orange, *Citrus aurantium* L., feeding-induced leaf volatiles repelled spider mites, thereby reducing damage (Agut et al. 2015). Tolerance strategies may also reduce negative impacts on fitness and yield (Stowe et al. 2000). For example, in navel oranges (*Citrus sinensis*), abscission was higher for fruit infested by thrips (*Pezothrips kellyanus*, Bagnall [Thysanoptera: Thripidae]) (Planes et al. 2014). Preferential abscission of damaged fruit may be a tolerance strategy in which the plant, at relatively low energetic costs, initiates a very large number of tiny fruits and then eliminates those that are damaged to divert major resource investment to the development of undamaged fruit (Goren 1993). Understanding the ability of the crop plant species to respond to herbivory can help to determine appropriate pest intervention strategies (Stout 2013, Peterson et al. 2016).

Citrus is a major agricultural crop worldwide and a high-value crop in California, United States, supporting a >$2 billion industry (Dreistadt 2012, USDA 2017). Citrus systematics is complex, with many of the designated species admixtures and interspecific hybrids containing various clonally propagated cultivars (Swingle 1943; Tanaka 1954; Hodgson 1967; Scora 1975; Barrett and Rhodes 1976; Federici et al. 1998; Barkley et al. 2006; Velasco and Licciardello 2014; Wu et al. 2014, 2018; Wang et al. 2018). Citrus production in California has changed substantially in recent years, with a sharp increase in acreage of citrus marketed as 'mandarins', especially *Citrus reticulata* and *Citrus clementina* species (CDFA and CASS 2018, CDFA 2018). 'Tango' and 'W.

Murcott Afourer' cultivars are the two most common *C. reticulata* cultivars grown in California, together representing nearly two-thirds of the total *C. reticulata* acreage (CDFA and CASS 2018). 'W.Murcott Afourer' also referred to as 'W. Murcott' or 'Afourer' or 'Delite' was imported to California from Morocco in 1985, and 'Tango' produced as part of the University of California Riverside (UCR) breeding program from a mutation induced by irradiating 'W. Murcott Afourer' budwood (UCR 2018).

A key pest of citrus in California is the endemic fork-tailed bush katydid (*Scudderia furcata* Brunner von Wattenwyl [Orthoptera: Tettigoniidae]) (Dreistadt 2012, Ferguson and Grafton-Cardwell 2014, Grafton-Cardwell et al. 2017). Katydids feed directly on young orange fruit, causing fruit to abscise or creating scars in the rind that persist to harvest. In the absence of fruit, they will also feed on the leaves of orange trees but it is only the direct fruit scarring that is of economic importance as it causes the fresh market fruit to be downgraded, resulting in nearly complete loss of fruit value. With saltatorial hindlegs and relatively large mouthparts, fork-tailed bush katydid nymphs are mobile and can damage many fruits in a short amount of time.

As a result, their advised treatment threshold is low (one katydid found in a 3-min search; Grafton-Cardwell et al. 2017). With no economically viable biological control options, fork-tailed bush katydids are commonly controlled with organophosphate or pyrethroid insecticides, which can be disruptive to natural enemies (Grafton-Cardwell 2015). Fork-tailed bush katydids are managed in California as part of a year-round Integrated Pest Management program established from extensive experience and field experiments primarily in sweet oranges (*C. sinensis*) including many 'Navel' and 'Valencia' cultivars, that has not yet been modified to accommodate mandarins (Grafton-Cardwell et al. 2017).

A previous ecoinformatics study analyzing sampling data from 202 commercial citrus groves for 1–10 yr in the main production region of California suggested that katydid scarring is much lower in 'Tango' and 'W. Murcott Afourer' (*C. reticulata*) mandarin cultivars, and also lower in 'Clemenules' (*C. clementina*) mandarins, than in *C. sinensis* sweet oranges, despite similar post-petal fall field densities of these pests in mandarin and orange fields (Cass et al. 2019). These observations raise the question of whether fork-tailed bush katydids are economic pests in *C. reticulata* mandarin cultivars. Several hypotheses were suggested to explain why katydid scarring is lower in mandarins than oranges, despite similar katydid field densities. These included: 1) a feeding aversion by the katydids to mandarin fruits, 2) a stronger expression of preferential abscission of damaged fruit by mandarins than by oranges, 3) developmental recovery of the scarred tissue in mandarins to regenerate the damaged tissue, and 4) different scar morphology on mandarins due to different feeding, or growth of the scar tissue, such that it is being misclassified at harvest as something other than katydid scarring. We report a series of field experiments to test these hypotheses, measuring katydid damage and fruit abscission and scarring in response to katydid damage and by simulating katydid bites with artificial damage. The experiments were conducted on *C. sinensis* 'Parent Washington Navel' sweet navel oranges (herein '*C. sinensis*') and *C. reticulata* 'Tango' mandarins (herein '*C. reticulata*') to observe response differences between these representative citrus cultivars.

Materials and Methods

Field Experiment Site

Field experiments were conducted at the Lindcove Research and Extension Center (LREC) in Petal Fall District 1 of Tulare County, California, United States. In 2017, experiments were run in a 'Parent Washington Navel' sweet orange block (*C. sinensis*) and an adjacent 'Tango' mandarin block (*C. reticulata*). The 1.57 ha *C. sinensis* block with C35 rootstock was planted in 1992 at 5.5 × 5.5 m spacing. The 1.53 ha *C. reticulata* block with Carrizo rootstock was planted in 2010 at 3.1 × 5.5 m spacing. The Tulare County Agricultural Commissioner declared petal fall in this district on 21 April 2017. Petal fall is the annual date on which an estimated 75% of the petals have dropped from the trees in the growing district and restrictions on the use of pesticides potentially toxic to bees are lifted in citrus groves. Here, we have reported experimental dates as days from petal fall rather than calendar dates, as a way to normalize the phenology among growing years and locations for generalization of the results.

Fork-Tailed Bush Katydid Collection and Rearing

Fork-tailed bush katydids aged first to third instar (most estimated to be second instar; Grafton-Cardwell 2017) were collected from two commercial citrus blocks in Fresno County, California, United States, 3 d prior to petal fall in 2017. The katydids were collected by coaxing them to jump up inside 1-liter plastic containers, which could then be closed without injury to the katydid, and then maintained as a colony indoors at room temperature until use in experiments. The colony was kept in two large bug dorms (BugDorm-2400F Insect Rearing Tent, L75 × W75 × H115 cm, nylon netting, MegaView Science, Taiwan) each with a small potted orange tree, water vials, and bouquets of untreated leaves, flush, fruit, and flowers from the two experimental blocks, changed approximately every 5 d. The katydids were observed to feed and molt, suggesting these rearing conditions to be adequate for their growth. The small potted orange trees were replaced once during the rearing period when the leaves had been extensively chewed.

Katydid Herbivory Experiments

Four independent but similar katydid herbivory field experiments were conducted in 2017, at two time intervals on each of the two citrus species: 'early', in the first 2 wk after petal fall (starting on days 6–8 after petal fall for *C. sinensis* and on petal fall and on days 1–2 after petal fall for *C. reticulata*) and 'late', 4–5 wk after petal fall (starting day 20 after petal fall for *C. sinensis* and day 27 after petal fall for *C. reticulata*). The different experimental start times for the two citrus species reflected their different phenology (flowering times). Individual fork-tailed bush katydid nymphs were caged on terminal clusters of fruit and leaves (approximately 20 cm of terminal branch length; the position of the bag closure was marked with wire and an aluminum identifying tag) using 19-liter (5 gallon) mesh paint strainer bags secured around the branches with wire. An equal number of no-katydid control cages was included on each tree. Experimental trees were distributed randomly across the western third of the field. Experimental and control branches were chosen at random within each tree section, interspersed around the full perimeter of the tree within 2 m from ground level. Sixty 'early' and 40 'late' cages were set up per treatment (control, katydid) within each citrus species grove, with five control and five katydid cages per tree. The katydid treatment was one katydid nymph released per cage and the control was a cage with no katydid. Some cages were excluded due to handling errors such as the bag having torn open or the wire coming loose, or if all fruits abscised prior to fruit assessment. Cages in which the katydid died and the matching control cage replicates were also excluded, because the length of exposure to the katydid was unknown. This left 51 control and 51 katydid in the *C. sinensis* 'early', 40 control and 38 katydid in the *C. sinensis* 'late', 53 control and 55 katydid in the *C. reticulata* 'early', and 26 control and 23 katydid in the *C. reticulata* 'late' replicate cages per experiment.

After 5 d, the cages were removed and katydid status was noted (alive/dead). Each fruit was labeled on the branch or petiole with an approximately 3 mm wide strip of label tape (FisherBrand). The numbers and sizes of the fruit are presented in Table 1. The flowers were not tagged as there was only 1 in the *C. sinensis* and 14 in the *C. reticulata* 'early' experiments. Fruit that abscised into the cage during the 5-d confinement interval or between cage removal and tagging were excluded, as many were too desiccated to assess damage levels.

The tagged fruits 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; 2, extensive surface-level chewing or many overlapping shallow cuts; or 3, one or more deep, chewed holes or majority of fruit consumed (Fig. 1). The individual shallow cuts were often observed to be chevron-shaped or paired, as expected from a single 'bite' with the paired mandibles. When a fruit had more than one level of damage, only the most severe damage level was recorded. We were careful to inspect and handle all control fruit in the same manner as was done for the katydid-exposed fruit, to limit the possibility that handling could also affect abscission. Additional notes were made about non-chewing damage such as deformities or bruises that could affect fruit development appearance.

We also recorded damage to other plant tissues on which the katydids could have been feeding. The presence/absence of chewing damage on the stigmas and/or styles (Fig. 1) was recorded in the two 'early' experiments where some fruit still had these tissues attached and were not desiccated. In the two 'late' experiments, the presence/absence of deep bite marks on the floral disc (Fig. 1) was recorded if this tissue was still visible between the smaller-sized fruit and sepals. Each leaf was categorized by leaf color as a mature leaf (dark green) or new flush (light green) and examined for evidence of feeding (slits, irregular holes, or missing areas on the leaf surface, middle or edges). The numbers of these tissues enclosed per cage are listed in Table 1.

Abscission of the tagged fruit was monitored at approximately monthly intervals until harvest. Some fruits were censored due to accidental damage to the branch or petiole during the interim assessments and an inadvertent pruning in the *C. sinensis* grove. The retained fruits were harvested when fully developed at 257 d after petal fall for *C. sinensis* and 284 d after petal fall for *C. reticulata*, and scar presence/absence was recorded. The polar and equatorial diameters of the fruit were measured using wide-arm calipers, and the maximum length and width of any deeper scars were measured with digital calipers.

Artificial Damage Experiments

Two artificial damage experiments were conducted concurrently with the katydid herbivory experiments. In the katydid herbivory experiments, we could not rule out the possibility that katydids might feed on fruits non-randomly, depending on some fruit characteristic that could be linked to the likelihood of subsequent abscission. This motivated us to complement the katydid herbivory experiment with simulated herbivory experiments where we could randomize the assignment of treatments to individual fruit and generate standardized damage levels. These experiments were conducted at 26 d post-petal fall in *C. sinensis* and 33 d post-petal fall in *C. reticulata*. In each grove, 150 fruits were tagged with aluminum tags secured loosely

Table 1. Descriptive statistics of experimental setup

	Katydid herbivory experiments												Artificial damage experiments					
	C. sinensis 'early'			*C. sinensis* 'late'			*C. reticulata* 'early'			*C. reticulata* 'late'			*C. sinensis*			*C. reticulata*		
	Total	Mean ± SD	Range	Total	Mean ± SD	Range	Total	Mean ± SD	Range	Total	Mean ± SD	Range	Total	Mean ± SD	Range	Total	Mean ± SD	Range
Number of fruit initially enclosed per cage[a]	–	9.1 ± 4.6	2–25	–	2.7 ± 1.5	1–7	–	14.5 ± 7.6	3–54		6.0 ± 2.8	2–13	–	–	–	–	–	–
Number of fruit tagged	577	5.7 ± 3.1	1–19	138	1.8 ± 1.1	0–4	1,108	10.5 ± 6.6	0–47	224	4.6 ± 2.3	1–12	150	15 ± 3.6	(7–24)	150	7.7 ± 1.7	4–12
Equatorial diameters of tagged fruit (mm)[b]	–	6.2 ± 1.4	3–11	–	9.6 ± 2.4	3–17	–	4.0 ± 1.0	1–9		7.6 ± 1.8	4–13	–	–	–	–	–	–
Number of stigmas/styles	113	2.8 ± 2.7	1–12	–	–	–	52	2.2 ± 2.4	1–12	–	–	–	–	–	–	–	–	–
Number of floral discs visible	–	–	–	117	1.9 ± 0.9	1–4	–	–	–	224	4.6 ± 2.4	1–12	–	–	–	–	–	–
Number of mature leaves enclosed per cage	–	10.0 ± 6.2	0–32	–	10.6 ± 9.6	0–59	–	10.9 ± 5.9	2–40	–	12.2 ± 10.0	3–63	–	–	–	–	–	–
Number of new leaves enclosed per cage	–	8.2 ± 8.1	0–34	–	6.5 ± 10.0	0–53	–	3.9 ± 5.5	0–24	–	5.7 ± 6.4	0–22	–	–	–	–	–	–

[a] Calculated from the number of fruit tagged plus the number of fruit that abscised into the bag, or the number of stubs left by abscised fruit on the branch for the *C. reticulata* 'early' experiment.

[b] Measured upon removal of the cages using a clear plastic ruler for the 'early' experiments and with digital calipers for the 'late' experiments.

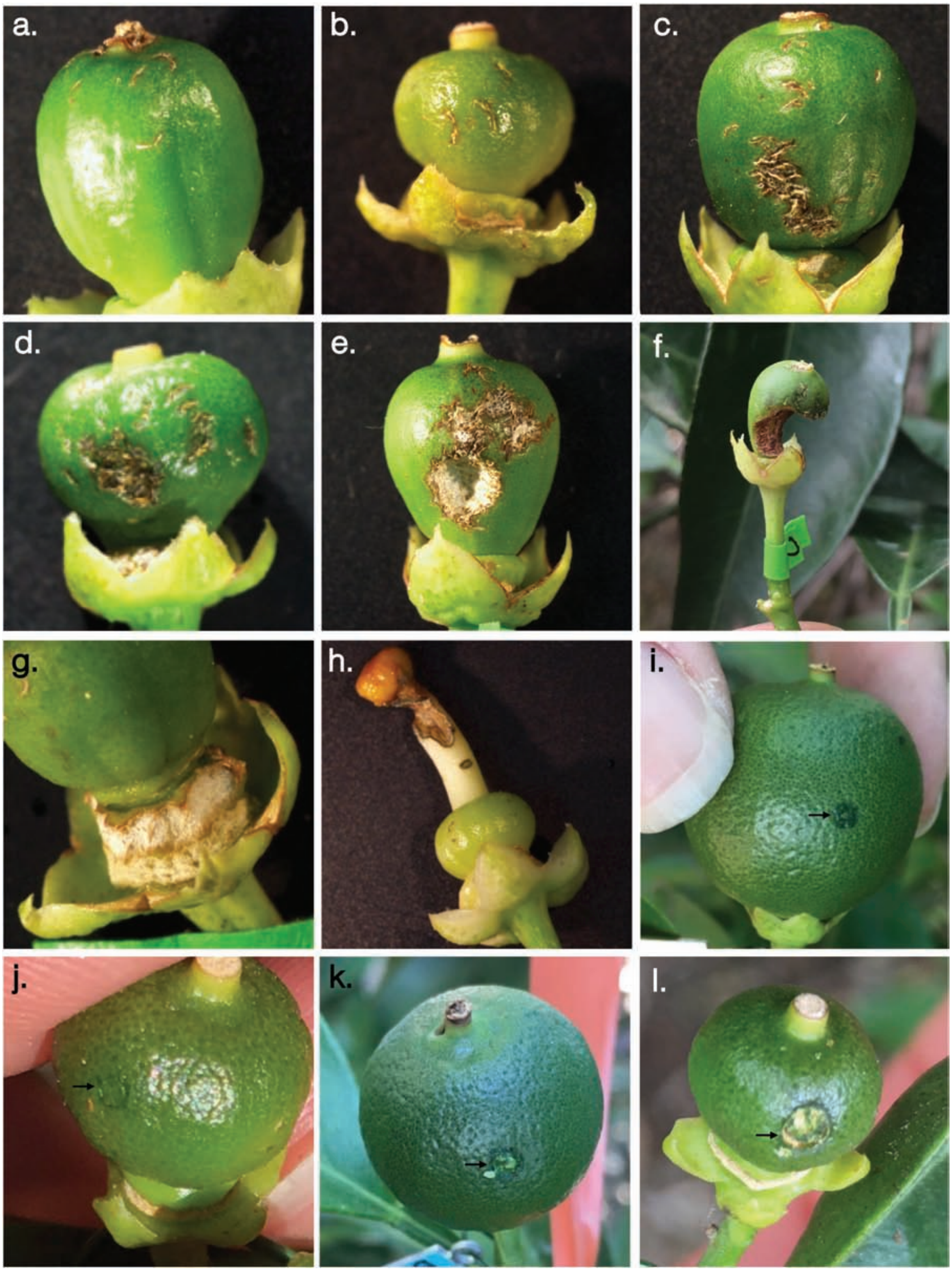

Fig. 1. Examples of katydid feeding damage (a–h) and artificial damage (i–l) to citrus fruit and floral tissue. Fruit damage classified as level 1 'individual linear or chevron-shaped cut/s' in (a) *Citrus sinensis* and (b) *C. reticulata.* Fruit damage classified as level 2 'extensive surface-level chewing' in (c) *C. sinensis* and (d) *C. reticulata.* (e, f) *Citrus sinensis* fruit damage classified as level 3 'one or more deep, chewed holes or majority of fruit consumed'. (g) Example of feeding damage to floral disc. (h) Example of feeding damage to the floral style. Artificial damage (i,j) with a 0.75-mm-diameter biopsy punch to create a shallow cut to *C. reticulata*, and (k,l) with a 1.5-mm-diameter biopsy punch and gouging with fine-point forceps to create an excised hole in (i,k) *C. sinensis* and (j,l) *C. reticulata.* Artificial damage on fruit in (i–l) indicated with an arrow.

around the petiole with wire, spread across five trees interspersed among the trees used in the katydid herbivory experiments. The 30 fruits per tree were divided among three treatments: 'heavy damage', 'light damage', and a 'no damage' control, for a total of 50 fruits per treatment. The 'heavy damage' fruits were punctured with a tubular, 1.5-mm-diameter biopsy punch to a depth of 1.7 mm and the tissue within the biopsied area scratched out with fine forceps. The 'light damage' fruits were punctured with a tubular, 0.75-mm-diameter biopsy punch to a depth of 0.5 mm, with the tissue within the punch not removed (Fig. 1). The tagged fruits were monitored and harvested as per the katydid herbivory experiments to assess abscission and scar development.

Statistical Analyses

Statistical analyses were performed in R version 3.5.1 (R Core Team 2018). For each of the katydid herbivory experiments, the effect of the predictor variable *katydid exposure* on the response variable *fruit damage level* was tested using one-way repeated ordinal regression (cumulative link mixed model [CLMM]) with *cage identifier* as a random effect, using the *clmm* function in the R package *ordinal* version 2018.8-25 (Christensen 2018) and the *Anova* function in the R package *car* version 3.0-2 (Fox and Weisberg 2011).

The effect of the predictor variable *katydid exposure* on the response variable *feeding damage to stigmas and/or styles* exhibited near or complete separation in both experiments where it was measured, so was tested with a Bayesian generalized linear mixed effect model with a binomial family distribution (number damaged, number undamaged per cage), normal family fixed effect prior (covariance matrix 9, 2), and *tree identifier* as a random effect, using the *bglmer* function in the R package *blme* version 1.0-4 (Chung et al. 2013). The effect of the predictor variable *katydid exposure* on the response variables *feeding damage to floral discs*, *feeding damage to mature leaves*, and *feeding damage to new leaves* were each tested using separate generalized linear mixed effect models (GLMM) with a binomial family distribution (number damaged, number undamaged per cage) and *tree identifier* as a random effect using the *glmer* function in the R package *lme4* version 1.1-18-1 (Bates et al. 2015).

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. Therefore, fruit abscission treatment group was assigned by the initial damage assessment score, regardless of exposure to katydids, meaning that some fruits with damage were from no-katydid control cages, and some fruits with no damage had been exposed to katydids. For these and the 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 mixed effects Cox survival models (Cox) in the R package *coxme* version 2.2-10 (Therneau 2018). In the katydid herbivory experiments, *cage identifier* was included as a random effect. In the artificial damage experiments, *tree identifier* was included as a random effect. Fruits that survived to harvest or that were accidentally pruned were right-censored.

The proportion of the fruit surface area with deep scar was calculated by dividing the area of the scar estimated as a rectangle, by the surface area of the fruit estimated as a spheroid (Klamkin 1971, constant = 1.5). The rectangular approximation may overestimate the area of irregular or circular scars, as it used the maximum length and width of the scar. The proportion of the fruit surface area scarred was compared between treatments with a *t*-test, excluding two fruits with scars that were traced back to non-katydid initial damage. The high levels of natural abscission in citrus present an experimental design challenge when trying to track fruit morphology from fruit set through to harvest, as all or almost all experimental fruit are lost in the first weeks of the experiment even starting with large cohorts. In the Results and Discussion, we pooled some scarring data from the few retained fruit across the different experiments as a way to still explore the trends.

Results

Citrus sinensis 'Early' Experiment

Katydids fed on the fruit generating superficial bites as well as deep, chewed holes (Fig. 2; CLMM, $\chi^2 = 80.6$, df = 1, $P < 0.0001$). All experimental fruits abscised by the seventh week post-petal fall and damage level decreased abscission rate (Cox, $\beta = -0.12$, $Z = -2.2$, $P = 0.03$). Katydids also fed on the stigmas and styles (Fig. 3; Bayesian GLMM, $\beta = 4.5$, $Z = 4.4$, $P < 0.0001$). They also fed on new leaves (GLMM, $\beta = 0.8$, $Z = 2.7$, $P = 0.008$) but did not consume mature leaves ($\beta = -0.7$, $Z = -1.5$, $P = 0.14$). Three katydids died during the experiment (proportion dead = 0.06).

Citrus sinensis 'Late' Experiment

Katydids fed on the fruit generating superficial bites as well as deep, chewed holes (Fig. 2; CLMM, $\chi^2 = 75.2$, df = 1, $P < 0.0001$). Damage level did not influence fruit abscission (Cox, $\beta = 0.14$, $Z = 1.8$, $P = 0.07$). Katydids also fed on the floral discs (Fig. 3; GLMM, $\beta = 2.0$, $Z = 2.6$, $P = 0.011$). They did not feed on leaves. Mature leaves had no damage in either treatment and damage to new leaves was not different between treatments (GLMM, $\beta = -0.9$, $Z = -0.7$, $P = 0.46$). One katydid died during the experiment (proportion dead = 0.03).

Citrus reticulata 'Early' Experiment

Katydids generated superficial feeding damage on the fruit (Fig. 2; CLMM, $\chi^2 = 49.3$, df = 1, $P < 0.0001$). In contrast to what had been observed in both of the experiments with *C. sinensis*, there was almost no severe fruit feeding damage. Damage level did not influence fruit abscission (Cox, $\beta = -0.16$, $Z = -2.0$, $P = 0.05$). Katydids did feed on the stigmas and styles (Fig. 3; Bayesian GLMM, $\beta = 2.7$, $Z = 2.2$, $P = 0.03$) but not leaves (GLMM, mature leaves $\beta = 0.2$, $Z = 0.4$, $P = 0.67$; new leaves $\beta = -0.3$, $Z = -0.7$, $P = 0.48$). Two katydids died during the experiment (proportion dead = 0.03).

Citrus reticulata 'Late' Experiment

Katydids generated superficial feeding damage on the fruit (Fig. 2; CLMM, $\chi^2 = 21.5$, df = 1, $P < 0.0001$). As observed in the *C. reticulata* 'early' experiment, there was almost no severe fruit feeding damage. Damage level did not influence fruit abscission (Cox, $\beta = -0.20$, $Z = 1.6$, $P = 0.11$). Katydids did feed on the floral discs (Fig. 3; GLMM, $\beta = 3.0$, $Z = 4.0$, $P < 0.0001$). They did not feed on leaves, with no damage to leaves in the control cages, only one mature leaf and two new leaves with damage across all the katydid cages. Thirteen katydids died during the experiment (proportion dead = 0.36).

Artificial Damage Experiments

Damage level did not influence fruit abscission in *C. sinensis* (Fig. 4, Cox, $\beta = -0.01$, $Z = -0.1$, $P = 0.96$) or *C. reticulata* ($\beta = 0.01$, $Z = 0.1$, $P = 0.92$). The heavy artificial damage resulted in katydid-like scars and the light artificial damage resulted in small scars in both in *C. sinensis* and *C. reticulata* (Fig. 5).

Scar Morphology

The deep holes resulted in scars with morphology typical of katydid scarring that covered approximately 1.37 and 1.24% of the fruit

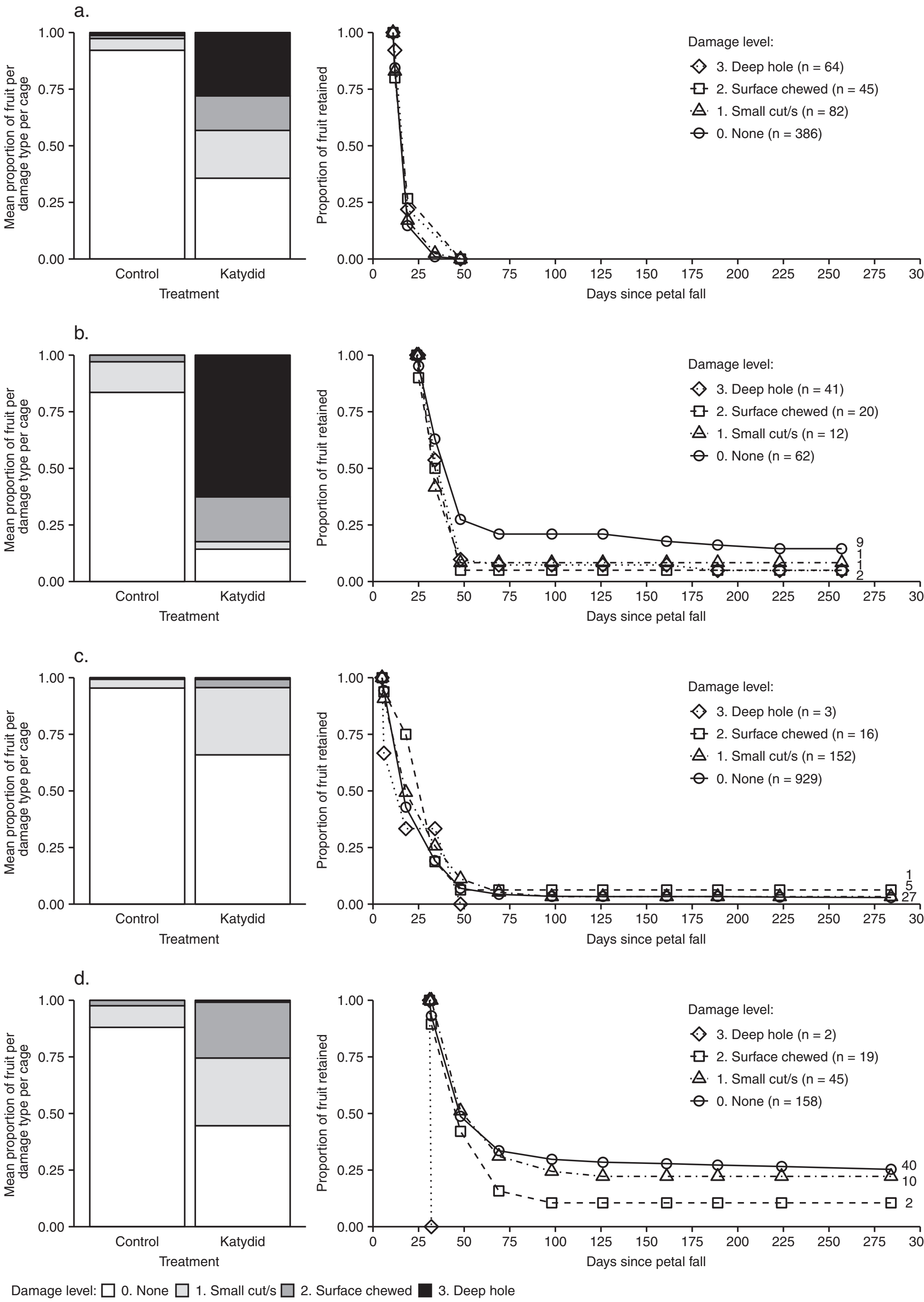

Fig. 2. Katydids generated significant deep and superficial feeding damage in (a) *Citrus sinensis* 'early' and (b) *C. sinensis* 'late' herbivory experiments, but mainly superficial damage in the (c) *C. reticulata* 'early' and (d) *C. reticulata* 'late' experiments (left). The damage in the control cages was likely mechanical damage or preexisting feeding damage from before the treatments were applied. Numbers in parentheses in the legend are the total number of fruit for each damage level across all experimental cages. Damage level only significantly affected retention in the *C. sinensis* 'early' experiment, where damaged fruit had a decreased abscission rate (left). Numbers at the ends of the censored retention curves are the number of fruit remaining at harvest.

surface area in the two retained *C. sinensis* fruits with this initial damage level (Fig. 5; Grafton-Cardwell et al. 2003). The five fruits with deep holes from the two *C. reticulata* experiments all abscised soon after petal fall, so we do not know the appearance of this damage on mature fruit.

The superficial damage was difficult to trace from the initial damage to harvest because the marks were small and indistinguishable from the background marks and scratches incurred from a variety of unknown sources. For initial surface chewing, the one retained *C. sinensis* fruit and one retained *C. reticulata* fruit from

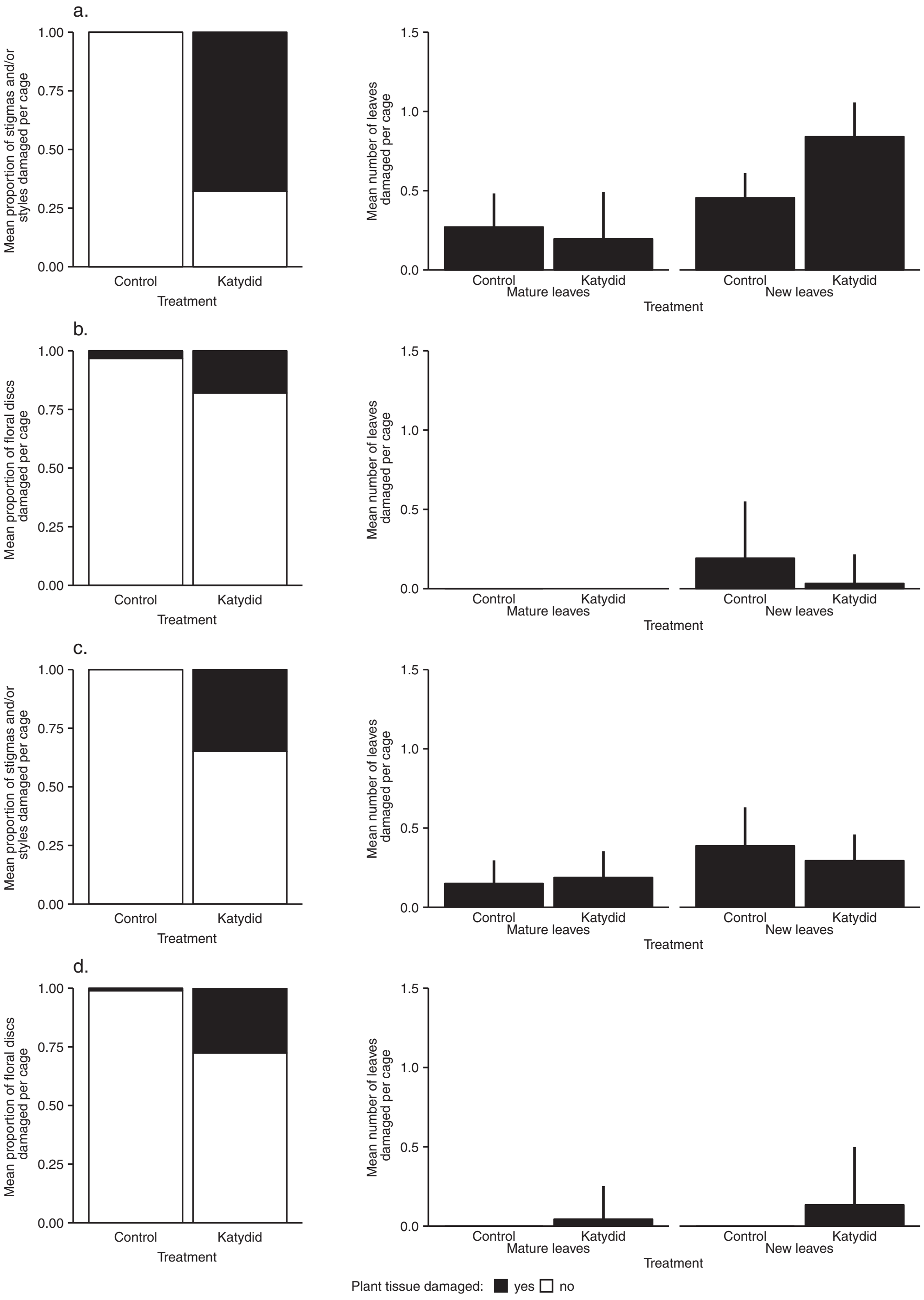

Fig. 3. Katydids fed on the floral tissue in the all experiments (right). Katydids fed on new leaves (left) in the (a) *Citrus sinensis* 'early' experiment, but not in the (b) *C. sinensis* 'late', (c) *C. reticulata* 'early', or (d) *C. reticulata* 'late' herbivory experiments. They did not cause significant feeding damage on mature leaves in any experiment.

katydid cages each had a shallow mark at harvest covering approximately 0.07 and 1.24% of the fruit surface, respectively. For initial shallow cut/s, there were no *C. sinensis* fruit retained from katydid cages. In the *C. reticulata* experiments, there were 12 fruits with initial shallow cut/s retained from katydid cages and at harvest they had minor scars covering 0.08 mean ± 0.12 SD % of the fruit surface area. This was not significantly different from the scarring levels in the 71 retained *C. reticulata* from katydid or control cages that had no initial damage (*t*-test, $t_{50.6} = 1.8$, $P = 0.86$; scarring covering 0.07 mean ± 0.36 SD % of the fruit surface area).

Discussion

Our experiments provide strong support for the feeding aversion hypothesis. Fork-tailed bush katydids fed readily on *C. sinensis* 'Parent Washington Navel' sweet oranges, chewing deep holes in the

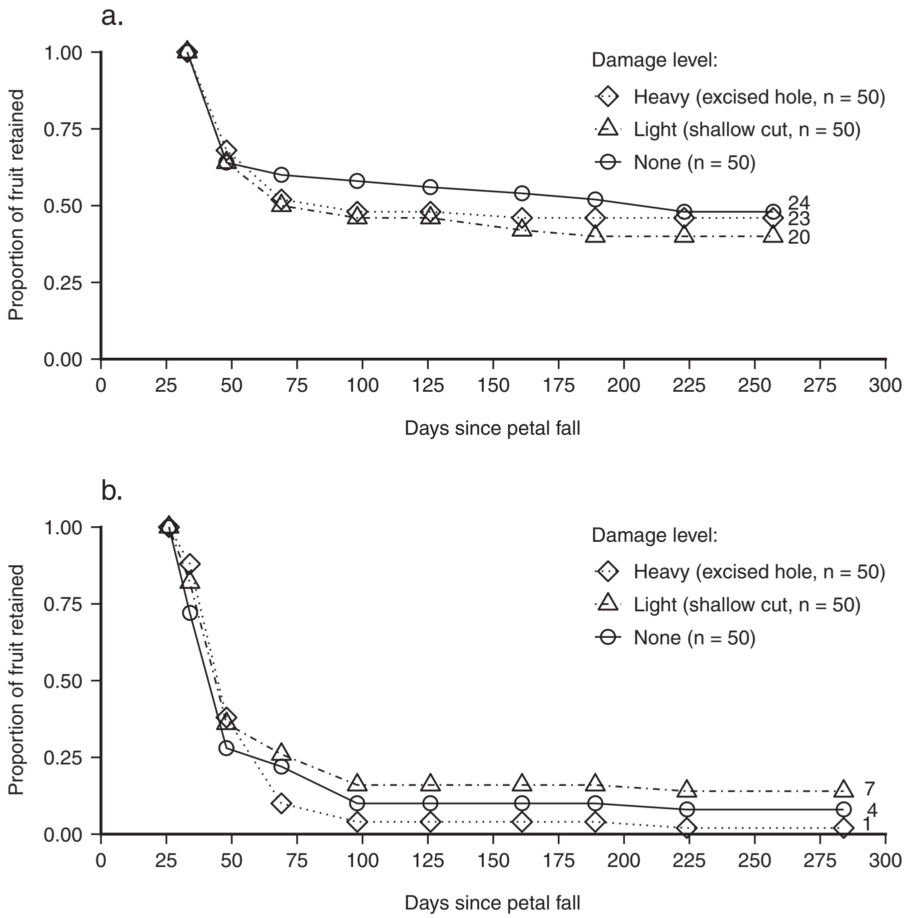

Fig. 4. In the artificial damage experiments, treatment level did not influence fruit abscission in (a) *C. sinensis* or (b) *C. reticulata.* Plots show the proportion of fruit retained of the total number of fruit per treatment, and the numbers at the end of the retention curves are the number of fruit remaining in each treatment at harvest.

fruit, but on *C. reticulata* 'Tango' mandarin fruit, they almost exclusively caused superficial cuts and, in the later experiment, some superficial chewing. We interpreted the chevron-shaped cuts seen on some fruits as the marks left when the katydid's paired mandibles sliced into the fruit, but the katydid then rejected the fruit upon making an initial tasting bite; whereas we interpreted the deep holes as the result of many repeated bites and ingestion of the fruit. This provides an explanation for the perplexing observation from Pest Control Advisor (PCA)-generated data of very low katydid scarring in *C. reticulata* mandarins compared to navel oranges, despite similar densities of the katydids in the weeks after petal fall: fork-tailed bush katydids seem to be present in *C. reticulata* trees, but are not eating the fruit.

The reduced feeding on *C. reticulata* fruits could be due to chemical or mechanical defenses by the plant. Are the insects tasting the fruit and then rejecting it because of a repellent flavor or toxic compound? Or are they unable to bite through the *C. reticulata* rind tissue? The rind appears from informal observations to be thinner but somewhat tougher than the rind of *C. sinensis* fruits. Mandarins could have molecular, anatomical, or biochemical factors that deter feeding, for example, 'Murcott' cultivar *C. reticulata* mandarins have been shown to have more oil cells in the flavedo than 'Star Ruby' *Citrus paradisi* MacFayden grapefruit (Shi et al. 2007), and have a different color and carotenoid compositions compared to oranges and grapefruit (reviewed in Tietel et al. 2011, Goldenberg et al. 2018). Additional research is needed to conduct mechanochemical profiling of the fruit, to examine whether these traits make the *C. reticulata* less palatable to other direct pests, and whether the protection is afforded only to fruits or to the whole plant.

There was higher mortality of katydids in the 'Tango' mandarin 'late' experiment than in the other three experiments, with one third of the katydids dying during the period of confinement on the branches. This could have been due to any number of differences in conditions and timing among the separate experiments, for example, there were slightly higher temperatures and lower humidity during the 'Tango' mandarin 'late' experiment than during the earlier experiments. (The mean temperature when the katydids were caged on the trees was 24.6°C [range 7.5–40.4°C] and the mean relative humidity was 42.8%, compared to mean temperature 17.0°C [6.6–31.1°C], 21.2°C [5.6–39.2°C], 16.6°C [6.2–30.0°C], and mean relative humidity 63.0, 52.5, and 55.8% in the *C. sinensis* 'early', *C. sinensis* 'late', and *C. recitulata* 'early' experiments, respectively; LNC Lindcove-01.P weather station.) An alternative possibility is that the katydids on *C. reticulata* died from starvation once there were no longer floral parts on which to feed. The only *C. reticulata* tissues on which we recorded the fork-tailed bush katydids to feed were the floral tissues: stigmas, styles, and floral discs, all of which become unavailable as the fruit increases in size (the floral parts abscise very quickly after petal fall, and as the fruit grows larger the floral disc is hidden between the developing fruit and sepals). We did not observe more leaf feeding by the katydids in the *C. reticulata* trees that might compensate for the lack of fruit feeding, although

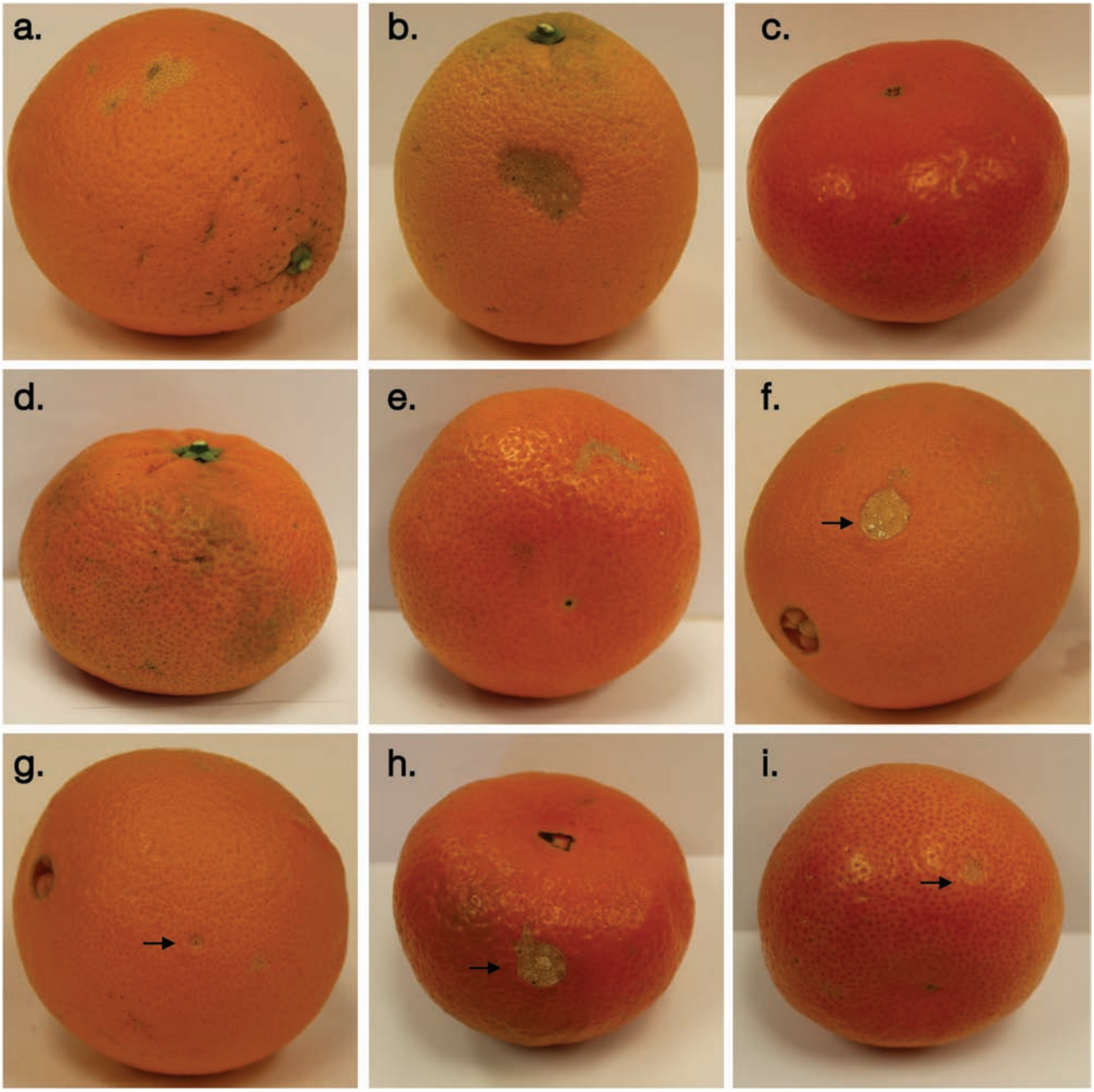

Fig. 5. Representative examples of morphology of scars on the retained fruit from cages with fork-tailed bush katydids or with artificially induced feeding damage: (a) *C. sinensis* fruit in the 'late' experiment with initial surface chewing ('level 2 damage'); (b) *C. sinensis* fruit in the 'late' experiment with initial deep chewed holes ('level 3 damage'); (c) *C. reticulata* fruit in the 'early' experiment with initial shallow cut/s ('level 1 damage'); (d) *C. reticulata* fruit in the 'late' experiment with initial shallow cut/s ('level 1 damage'); (e) *C. reticulata* fruit in the 'late' experiment with initial surface chewing ('level 2 damage'); (f) *C. sinensis* 'heavy' artificial damage (excised hole); (g) *C. sinensis* 'light' artificial damage (superficial cut); (h) *C. reticulata* 'heavy' artificial damage (excised hole); (i) *C. reticulata* 'light' artificial damage (superficial cut). Scarring on artificially damaged fruit in (f–i) indicated with an arrow.

note that the overall leaf feeding observed in the cages was very low, even in *C. sinensis* where folivory is known to occur. If the fork-tailed bush katydids are unable to complete development on *C. reticulata*, this *Citrus* species may be an ecological 'sink' for katydid populations that develop on nearby susceptible 'source' crops (Dias 1996). Alternatively, they may be sustained in *C. reticulata* with low levels of herbivory that we were not able to detect with the methods used in this study.

Preferential abscission of damaged fruit does not appear to contribute substantially to the difference in final scarring rates between oranges and mandarins. In the both *C. reticulata* herbivory experiments and in the *C. reticulata* artificial damage experiment designed specifically to test this hypothesis, there were only non-significant trends. The high overall abscission rates, especially in *C. sinensis* (Goren 1993), and the low frequency of heavy feeding damage in *C. reticulata* limited the power of these experiments. It is possible that more pronounced differences would have been observed with many more replicate fruit. Although the artificial damage using biopsy punches provided the advantage of allowing us to assign treatments to fruit randomly, it had the disadvantage of simulating only the mechanical aspects of katydid damage, and lacked elements, such as saliva and/or microbes, that might also be associated with natural katydid feeding and that might affect abscission.

Our assessment of the third and fourth hypotheses concerning scar development was limited by the effects of the feeding preference and abscission that acted earlier in the experiment to reduce the final sample sizes of harvested fruit. It is inherently difficult to track early fruit damage through to harvest in trees with such high natural abscission levels; even starting with a large cohort of young experimental fruit, all or almost all of them abscise. The main type of feeding damage in *C. reticulata* (superficial cuts or chewing) did not result in the typical round scars from deeper chewed holes in *C. sinensis*. The rind damaged in the artificial damage experiment did not recover; in particular, when we used a biopsy punch to simulate katydids chewing a deep hole into the rind of a 'Tango' mandarin, the resulting scar was deep and scabby, just as observed when katydids chew deep holes into navel oranges. Thus, the extreme rarity of such scars in hundreds of commercial harvests (Cass et al. 2019) suggests that such damage did not occur (or that heavily damaged fruits abscised before harvest), and do not support the hypothesis that the fruits were capable of healing the damaged tissue. We did observe some smaller, superficial, irregular-shaped scars in *C. reticulata*, providing further evidence against the hypothesis of damage recovery. However, we could not definitively trace the small, superficial scars back to the original katydid damage, and they were not easily distinguishable from the background damage incurred by initially undamaged fruit. More work is needed to help determine if irregular scars do result from superficial damage, how likely they are to cause downgrading of the fruit and/or be misclassified as wind or cutworm damage.

Overall, the picture that emerges from these experiments is that heavy feeding damage by fork-tailed bush katydids is extremely rare in 'Tango' and 'W. Murcott Afourer' cultivars of *C. reticulata*, and the rare, heavily damaged fruit often promptly abscise in response. Fork-tailed bush katydids do cause superficial feeding damage in these *C. reticulata*, but this damage is likely of minimal, if any, economic consequence at harvest. One limitation of these experiments is that it is difficult to determine whether the smaller trends observed may still have substantial impact on final scarring frequencies when scaled up to the numbers seen in commercial production. Some caution is also needed when interpreting the damage rates, as feeding behavior may have been affected by confinement in cages. Another limitation is that we cannot compare the results directly between the two citrus species or determine any causal effect of citrus species on the tested response variables, as the experiments were performed in separate, albeit adjacent, groves. As we tested only two time-windows after petal fall, it is possible that significant damage might have occurred during other periods. However, this seems unlikely, as the results from the database suggest that 'Tango' and 'W. Murcott Afourer' cultivars are nearly free of katydid scarring damage at harvest across a wide range of locations (Cass et al. 2019). We only tested one cultivar of each citrus species, so caution should be taken when extrapolating the results to other cultivars. Similar experiments could be conducted in *C. clementina* mandarins to see if the same mechanism is behind the relatively low katydid scarring observed in the 'Clemenules' cultivar (Cass et al. 2019).

This project has taken an interesting trajectory, beginning with an unconventional, 'big data' observational approach to tackle the difficult challenge of adapting pest management guidelines to a new crop species (Cass et al. 2019). The ecoinformatics dataset raised unexpected questions about the pest and host plant biology: what do fork-tailed bush katydids eat? And how do mandarin trees defend themselves against herbivory? These natural history questions brought us from 'big data' to more traditional field experimental methods. Pairing these complementary observational and experimental approaches has been a powerful approach to generate and test hypotheses in this agricultural system. With the combined support of these experimental results and the previous observational database results covering hundreds of commercial groves and growing conditions, we suggest that 'Tango' cultivar and, based on the database analysis, also 'W. Murcott Afourer' cultivar *C. reticulata* mandarins have innate resistance to fork-tailed bush katydids. The typical, common petal fall treatments of pyrethroids and organophosphates for katydids in these cultivars can therefore be eliminated in favor of effecting the plant's natural defenses. This would constitute a substantial reduction in insecticide use in California across the ~10,000 ha of these cultivars commercially grown.

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