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Vitis vinifera (Vitales: Vitaceae) as a Reproductive Host of *Spissistilus festinus* (Hemiptera: Membracidae)

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Abstract

Feeding by the three-cornered alfalfa hopper, *Spissistilus festinus* (Say) (Hemiptera: Membracidae) results in girdling of grapevine petioles and shoots. Its significance as an economic pest of grape has increased since it was shown to transmit Grapevine red blotch virus (GRBV) in a greenhouse study. However, the status of grapevines as a reproductive host for *S. festinus* remained undetermined. Adult *S. festinus* were caged onto three regions of the grapevines: apical shoot, green shoot, and dormant cane. Their ability to reproduce was determined by weekly destructive sampling for 7 wk. Successful oviposition and nymphal emergence were observed on apical and green shoots, but not on dormant canes. However, insect development beyond the second nymphal instar did not occur. Knowledge of *S. festinus* reproduction on grapevines will be an important consideration in designing management guidelines to minimize the spread of GRBV in vineyards.

Key words: *Spissistilus festinus*, three-cornered alfalfa hopper, reproductive hosts, Grapevine red blotch disease

Sequential stylet punctures around petioles and shoots of its plant host during feeding by the three-cornered alfalfa hopper, *Spissistilus festinus* (Say) create a ring of necrotic tissue described as a girdle (Wildermuth 1915, Beyer et al. 2017). Girdling damage to grapevines, *Vitis vinifera*, was generally considered to have little economic significance. As a result, *S. festinus* was regarded as only an occasional grape pest (Smith 2013), and details of its biology, including its ability to reproduce on grapes, received little research attention. The pest status of *S. festinus* has increased dramatically following a report that determined it to be a vector of Grapevine red blotch virus (GRBV) in a greenhouse study (Bahder et al. 2016) and its association with the spread of grapevine red blotch disease (GRBD) in a California vineyard (Cieniewicz et al. 2018). GRBD poses a serious economic threat to the wine industry due to delayed fruit maturity, reduced sugar accumulation, and adverse impacts on secondary metabolites responsible for wine flavor, color, and aroma (Calvi 2011, Al Rwahnih et al. 2013, Sudarshana et al. 2015, Wallis and Sudarshana 2016, Blanco-Ulate et al. 2017).

The biology of *S. festinus* has been more broadly studied in relation to soybean, peanut, and alfalfa production in the southern United States, where its feeding and density of oviposition in plant material can result in economic damage (Wildermuth 1915, Beyer et al. 2017). Unlike most other treehoppers, *S. festinus* is multivoltine

in warmer regions (Mitchell and Newsom 1984, Wildermuth 1915, Beyer et al. 2017) and does not require a woody host for oviposition (Mueller and Dumas 1987, Wildermuth 1915). Some studies indicate that although *S. festinus* can feed on a wide range of herbaceous hosts, not all of their feeding hosts serve as reproductive hosts (Newsom et al. 1983, Wildermuth 1915). Here we report the results of a field study conducted to determine the status of *V. vinifera* as a reproductive host of *S. festinus*, an important consideration in developing a management approach for this insect and determining its role in the epidemiology of GRBV.

Materials and Methods

Vineyard Description

The experiment was conducted in a research vineyard located near Davis in Solano County, CA (38°31'18.4"N, 121°45'36.4"W, 14 m) from July to September 2017, a period when *S. festinus* can be seen feeding on grapes. The research vineyard block consisted of 10 rows with 20 staked 6-yr-old Cabernet Sauvignon (clone 8) vines per row. Vine spacing was 1.5 m × 3 m within and between rows, respectively, with a North to South orientation. No insecticides or fungicides had been applied to the grapevines prior to the start of the experiment. The vineyard was watered using furrow irrigation applied as needed.

Treatments and Experimental Design

The experiment had three treatments with 21 replicates each, arranged in a randomized block design. Nylon mesh paint strainer bags (3.8-liter) with elastic openings (#SB101, Master Craft) were used as cages placed around each treatment area. The three treatment areas included the 1) apical shoot, the growing tip to the fifth node; 2) the green shoot, 9th node to the 12th node; and 3) the dormant cane, 2-yr dormant wood. Twenty-one grapevines were randomly selected; all three treatments were set up on each of the 21 grapevines ($N = 63$ cages total). Adaxial and abaxial surfaces of grape leaves within each cage were gently brushed with a 10.2 cm flat paintbrush to remove any insects or spiders to prevent predation and/or competition for resources prior to applying the cage. Adult *S. festinus* were collected with sweep nets from alfalfa, *Medicago sativa*, fields near Davis, CA (38°32'0.9198"N, 121°48'8.5248"W, elevation 19 m; 38°32'25.4832"N, 121°46'44.9976"W, elevation 16 m; 38°30'22.6146"N, 121°45'27.3558"W, elevation 13 m). Five female and five male *S. festinus* adults were introduced into each cage, the elastic opening sealed with duct tape and a row of continuous staples applied as reinforcement. Adults were contained until their cages were removed for sampling.

Sampling

The experiment duration of 49 d was 2 wk longer than previous research determined to be sufficient in documenting development from egg to adult (Mitchell and Newsom 1984). Therefore, adult emergence would be expected in the experimental time frame if *V. vinifera* served as a good host for *S. festinus* development. Three randomly chosen grapevines were destructively sampled at 7-d intervals for 7 wk. The shoots and canes were cut just beyond the attachment points of the cage to the grapevine and transported to the laboratory where they were opened and inspected under magnification using a dissecting scope (MZ7.5, Leica Microsystems, Wetzlar, Germany). The numbers of live and dead *S. festinus* adults and nymphs found in each cage on each sampling date were counted. Nymphal stage, number of girdles, and plant structure where eggs were deposited were also determined. Emergence of *S. festinus* nymphs from oviposited eggs was considered the criterion for successful reproduction (Moore and Mueller 1976, Mueller and Dumas 1987).

Results

In total, 186 and 133 plant structure sites containing ovipositional slits were found on apical and green shoots, respectively (Figs. 1; 2A and B). Eggs were primarily laid in the bracts or buds of apical shoots (61%) with the remaining being laid in petioles (20%) or shoots (17%) (Fig. 2A). Only 2% were laid in the leaf vein and none were laid in the leaf blade. In green shoots, eggs were primarily laid in the petioles of green shoots (41%), followed by bracts or buds (28%) and shoots (16%) (Fig. 2B). Fewer eggs were laid in leaf veins (10%) or the leaf blade (5%).

No nymphs emerged, no oviposition was observed, and no adult *S. festinus* survived beyond the first week in any of the 21 cages enclosing dormant canes. Nymphs of *S. festinus* began to emerge from the apical shoots during the second week and from the green shoots during the third week after caging the adults (Fig. 3A and B). The total composition of emerged nymphs recorded in apical and green shoot cages present at the time each cage was removed was

79% first instars and 21% second instars. No third through fifth instar nymphs or newly emerged adults were found.

During the course of the 7-wk experiment, a total of 217 nymphs were counted in apical cages of which 62 (29%) were live and 155 (71%) were dead (Fig. 4A). In total, 194 nymphs were counted in green shoot cages of which 48 (25%) were live and 146 (75%) were dead (Fig. 4B).

Girdles were documented in each apical shoot cage sampled ($n = 21$) and in 20 of the green shoot cages sampled ($n = 21$), starting from the first week through the seventh (Fig. 5). The mean number of live adults diminished over the duration of the study, but live adults remained in the apical and green shoot cages throughout the experiment (Fig. 6).

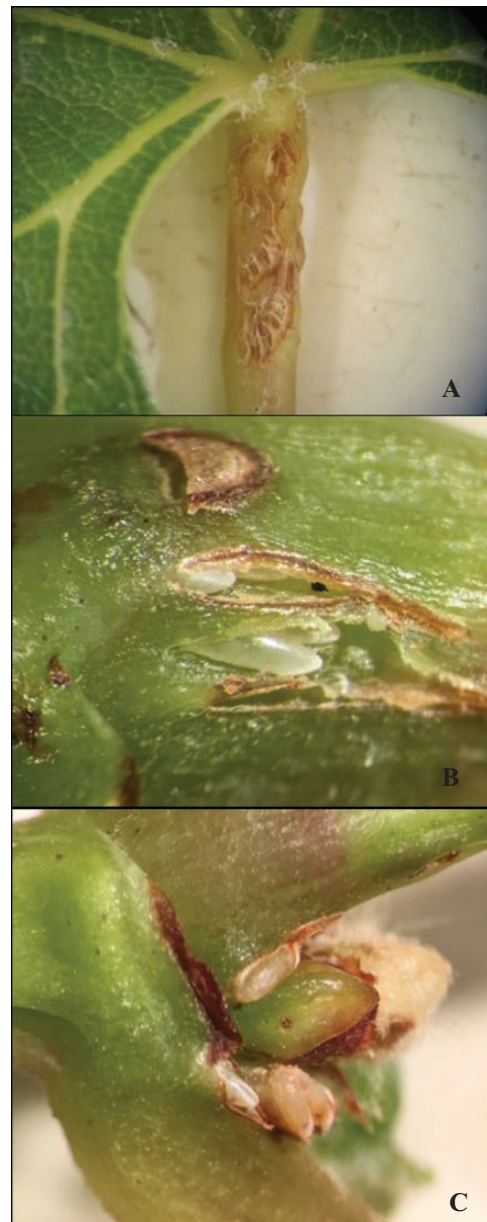


Fig. 1. *Spissistilus festinus* oviposition in (A) petiole, (B) shoot, and (C) bract/bud locations in apical and green shoots of grapevines.

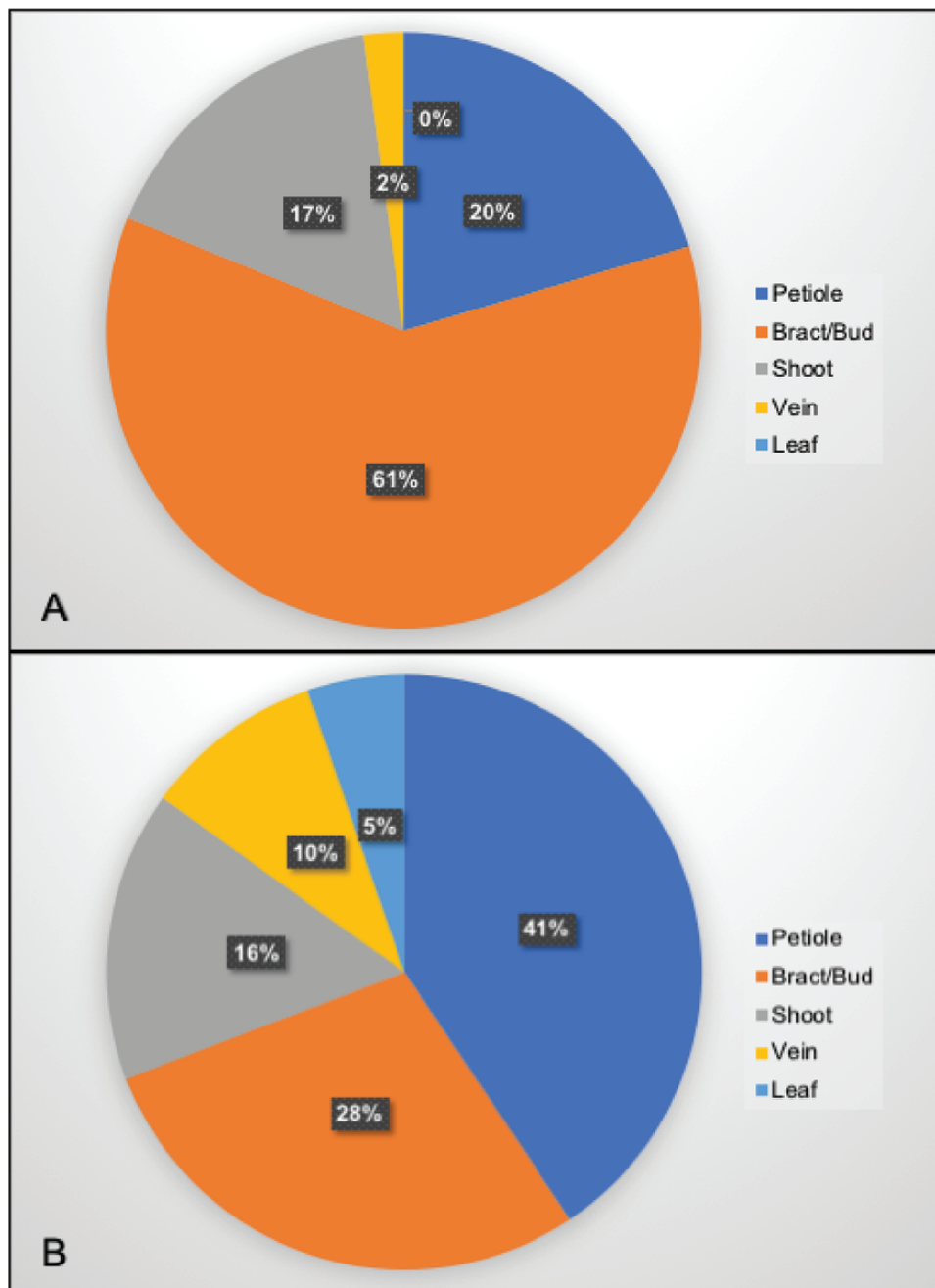


Fig. 2. Plant structure where *Spissistilus festinus* oviposition slits were found and frequency for (A) apical shoots, $n = 186$ and (B) green shoots, $n = 133$.

Discussion

Spissistilus festinus has a wide range of feeding and reproductive hosts with the best studied host species being herbaceous plants (Mueller and Dumas 1987, Wildermuth 1915). A number of trees and shrubs have also been reported as feeding hosts (Newsom et al. 1983, Wistrom et al. 2010); however, only wedgeleaf saltbush (*Atriplex truncata*), cocklebur (*Xanthium strumarium*), and apple (*Malus pumila*) have heretofore been reported as reproductive woody hosts of *S. festinus* (Moore and Mueller 1976, Mueller and Dumas 1987, Osborn 1911, Wildermuth 1915). Our finding that *V. vinifera* can serve as a reproductive host is particularly significant given its status as a vector of GRBV (Bahder et al. 2016).

Previous research has indicated that *S. festinus* prefers tender plant tissue over woody tissue for oviposition (Daigle et al. 1988, Wildermuth 1915, Beyer et al. 2017). Our results are in agreement with these studies, as we found no oviposition or nymph emergence from dormant canes, while both oviposition and nymph emergence were observed in the tender green tissue of apical and green shoots. *Spissistilus festinus* has been reported to overwinter both as adults and eggs on other woody and herbaceous hosts (Wildermuth 1915), although we have not determined to date whether later season oviposition in *V. vinifera* by *S. festinus* can result in overwintering eggs that could serve as a source in the vineyard the following spring. However, it might be expected that removal and disposal of canes

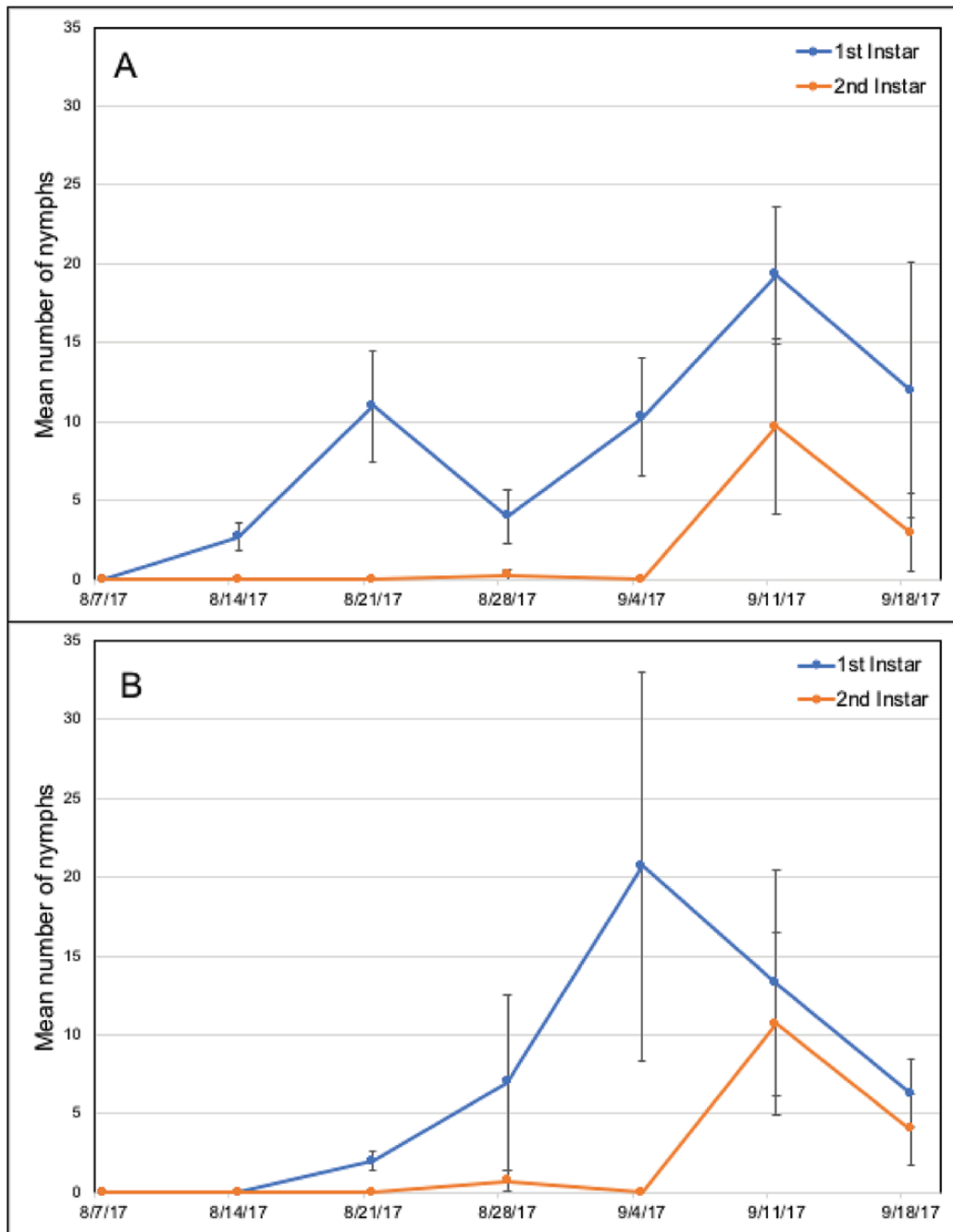


Fig. 3. Mean number (\pm SEM) of first and second instar *Spissistilus festinus* nymphs (live and dead combined) found in cages covering (A) apical shoots and (B) green shoots.

after winter pruning in commercial vineyards would reduce the number of overwintering eggs in any case.

We observed high *S. festinus* nymphal mortality in cages placed on both the apical and green shoots of grapevines. Mitchell and Newsom (1984) estimated the *S. festinus* generation time from oviposition to adult emergence to be 35 d. Our study spanned 49 d in which a full generation might be expected, and by the end of that

period no cage contained third through fifth instar nymphs, and only 18 and 24% of the total nymphs that emerged in apical and green shoots, respectively, reached the second instar. This finding suggests that while *V. vinifera* can be a feeding and reproductive host for *S. festinus* adults, it is not an ideal host for *S. festinus* nymphal development. During field sampling, we observed majority of the nymphs crawling on the cage material while very few were actively

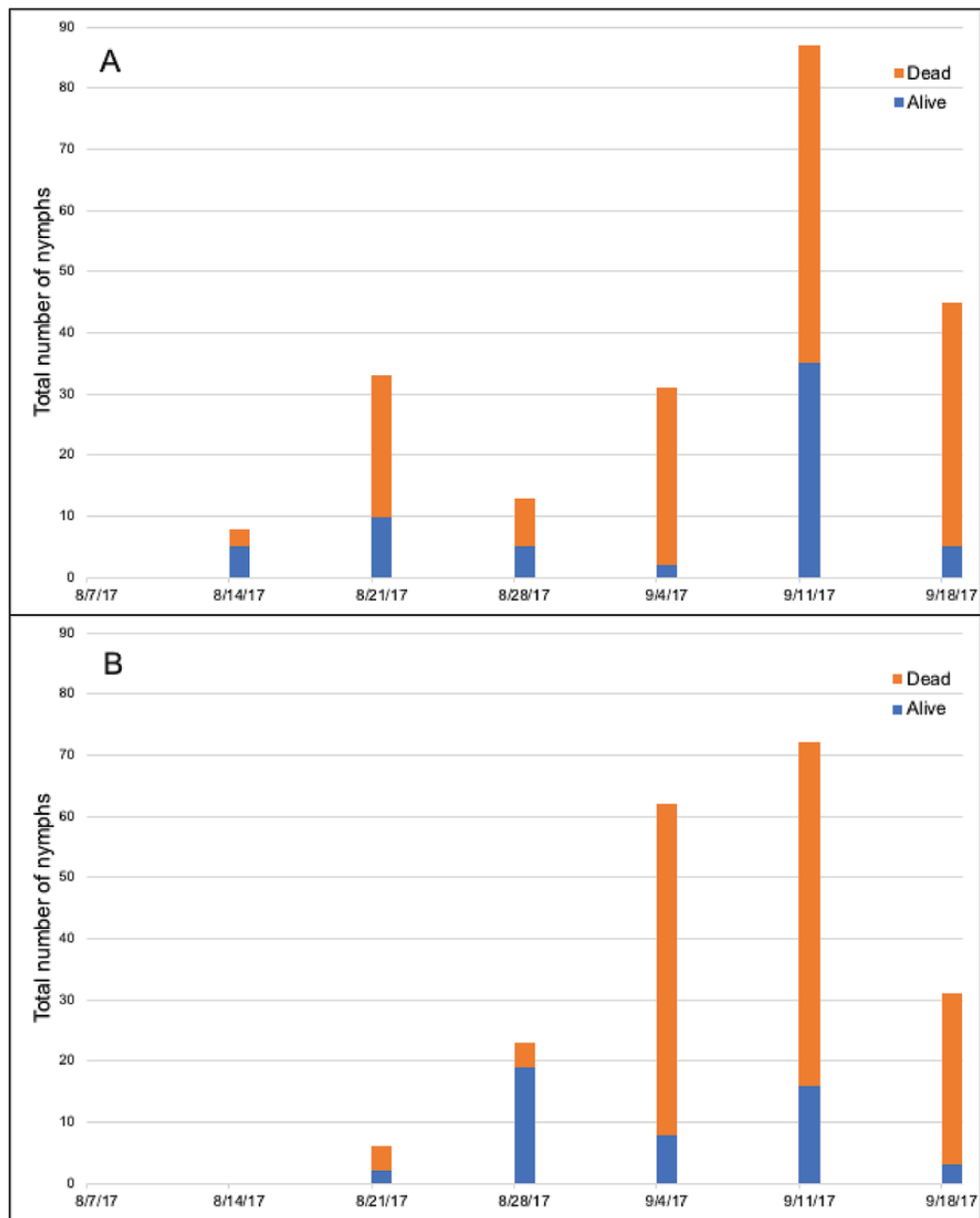


Fig. 4. Total number of live and dead *Spissistilus festinus* nymphs documented per sample date in (A) apical shoot cages, $n = 217$ and (B) green shoot cages, $n = 194$.

feeding on the grapevine. This behavior is similar to that previously described for *S. festinus* nymph emergence on apple trees. Lovett (1923) reported that after emerging the nymphs immediately drop from the trees to feed on ground vegetation. It is unknown whether this behavior is due to poor host suitability, difficulty in penetrating the woody plant material when feeding, or some other reason. This searching behavior in lieu of feeding, and the inability to drop to the

ground vegetation due to confinement in cages, may have contributed to the heavy *S. festinus* nymph mortality in our study. Future studies to clarify the role of *V. vinifera* and groundcover including resident vegetation and cover crops in the lifecycle of *S. festinus* in vineyards would be beneficial in the management of this insect species in the event it proved to be an epidemiologically significant GRBV vector.

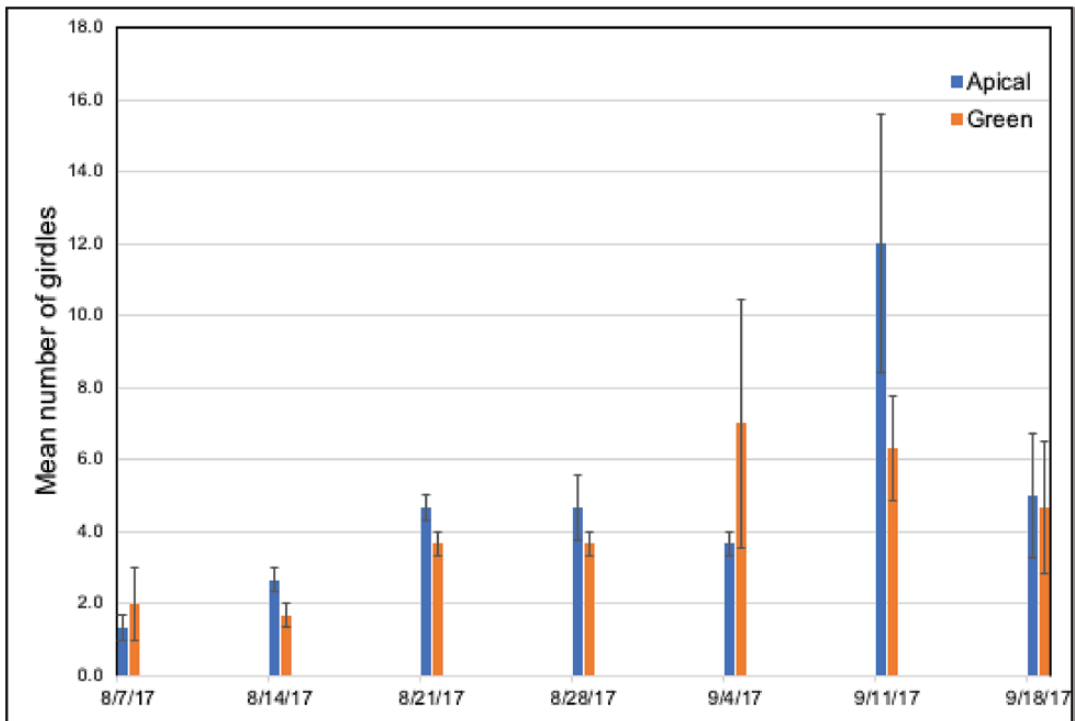


Fig. 5. Mean number (\pm SEM) of girdles documented per sample date from apical and green shoot cages.

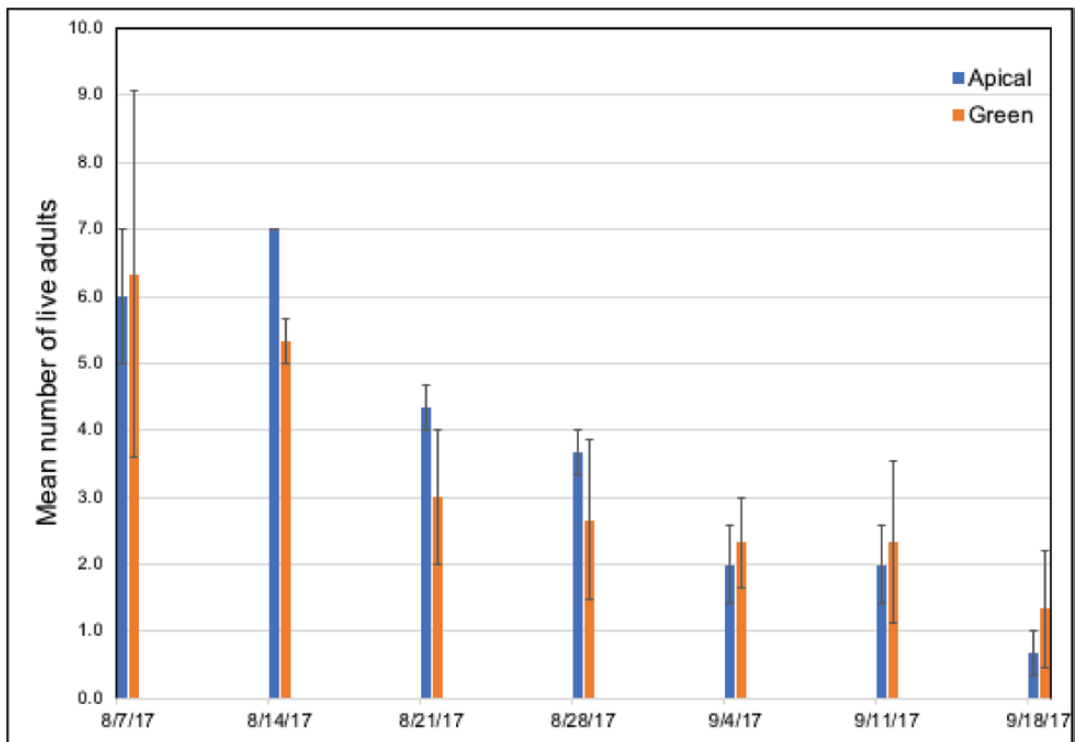


Fig. 6. Mean number (\pm SEM) of live *Spissistilus festinus* adults collected per sample date from apical and green shoot cages.

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