

# Absence of photoperiod effects on mating and ovarian maturation by three haplotypes of potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae)

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## ABSTRACT

We examined the effects of photoperiod on reproductive diapause of three haplotypes of potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), collected from three geographic locations: south Texas (Central haplotype), California (Western haplotype), and Washington State (Northwestern haplotype). Psyllids were reared from egg hatch to adult eclosion under short- and long-day conditions, to determine whether short days led to a lack of mating, delays in ovarian development, and accumulation of fat by female psyllids. Our expectation was that a reproductive response to short days, if present, would more likely be exhibited by psyllids of the northern-latitude haplotype (Northwestern) than by psyllids of the other two haplotypes. We also examined whether this species exhibited a photoperiod-controlled polymorphism in body size, as observed in other psyllid species, by comparing six body and wing measures of psyllids reared under short- and long-day conditions. Virtually 100% of females of each haplotype exhibited both egg maturation and mating at both long- and short-day conditions, providing no evidence that this species exhibits a photoperiod-induced reproductive diapause. Fat was present in most psyllids, although with higher probability of presence in short-day females than in long-day females. Photoperiod had no effect on body size. We found differences among haplotypes in body size, with psyllids from Washington State (Northwestern haplotype) having larger wings and longer tibiae than psyllids of the two southern populations. Our photoperiod results, combined with overwintering observations for this species and for other Triozidae, prompted us to hypothesize that potato psyllid—at least in the Pacific Northwest growing region—overwinters in a temperature-controlled quiescence rather than in a true diapause.

**Key Words:** reproductive diapause, ovarian development, overwintering, *Bactericera cockerelli*

## INTRODUCTION

The potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a serious pest of solanaceous crops in western North America, Mexico, Central America, and (as an introduction) in New Zealand (Wallis 1955; Teulon et al. 2009; Munyaneza 2012). Outbreaks of potato psyllid have been recorded in the United States since the early 1900s, leading to severe damage to potato, tomato, and pepper crops (Wallis 1955; Pletsch 1947). Potato psyllid is a vector of a phloem-limited bacterium that is associated in solanaceous crops with mortality of plants, lowered yields, and disorders of the harvested

product (Munyaneza et al. 2007; Hansen et al. 2008; Liefiting et al. 2008, 2009; Munyaneza 2012). In commercial potato fields, zebra chip disease of tubers has caused severe economic losses to potato growers since the first appearance of the disease in Mexico during the mid-1990s (Secor et al. 2009). Zebra chip disease has since caused extensive damage to potatoes in Central America, New Zealand, and in the Southwestern and Midwestern potato-growing regions of the United States (Munyaneza et al. 2007; Liefiting et al. 2008; Secor et al. 2009; Teulon et al. 2009; Munyaneza 2012). In 2011, zebra chip was

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detected for the first time in Washington, Idaho, and Oregon (Crosslin et al. 2012a, 2012b), comprising the three growing regions responsible for more than 50% of U.S. potato production.

Preventing zebra chip disease requires control of potato psyllid, which in turn requires understanding the vector's biology throughout its geographic range. There has been a great deal of historical uncertainty about the overwintering biology of potato psyllid, and much of this uncertainty continues today. It is likely that potato psyllid migrates northwards from its southern "breeding areas" (Romney 1939) in northern Mexico and the southwestern U.S., prompted by the onset of hot, dry conditions in those southern regions beginning in late spring and early summer (Romney 1939, Pletsch 1947, Wallis 1955). The northward dispersal appears to extend as far as southern Canada, and is thought to have been the source of outbreaks by potato psyllid along the Rocky Mountain potato- and tomato-growing regions during the outbreak years of the 1900s (Romney 1939; Wallis 1955). The psyllid disappears from southern breeding regions during the hot conditions of summer, reappearing (often on non-cultivated Solanaceae) in autumn only after cooler and wetter conditions have arrived (Romney 1939). The source of psyllids reappearing in autumn is unclear (Romney 1939, Wallis 1955). The fate of psyllids that have colonized the northern latitudes during late summer is also not known. The historical consensus has been that potato psyllids that have colonized these colder northern regions are unlikely to survive these regions' winter conditions (Wallis 1955; Pletsch 1947).

Our understanding of overwintering has become even more uncertain with the realization that potato psyllid is actually a complex of several genetically distinct populations, or haplotypes (Liu et al. 2006; Swisher et al. 2012, 2014). Four haplotypes are currently known (Swisher et al. 2013b, 2014), with centers of concentration in the western U.S. and Baja California (Western haplotype), southern Texas northwards along the Rocky Mountains and southwards into Mexico and Central America (Central haplotype), New Mexico and Colorado (Southwestern haplotype), and the Pacific Northwest (Northwestern haplotype). Three

haplotypes (Central, Western, Northwestern) can be found in potato-growing regions of Washington, Oregon, and Idaho in summer (Swisher et al. 2013a), with the Central and Western psyllids possibly arriving in the region by dispersal (Munyaneza et al. 2009). In contrast, the Northwestern haplotype is present year-round in the Pacific Northwest study area (Murphy et al. 2013; Swisher et al. 2013c), where it overwinters in association with a perennial nightshade, *Solanum dulcamara* L. (Solanaceae).

Psyllids of temperate regions often overwinter as adults in reproductive diapause, a state in which development has been suspended until environmental conditions are more suitable (Lauterer 1982, 1999; Hodkinson 2009). In obligate univoltine psyllids, including many *Cacopsylla* (Psyllidae) (Lauterer 1999; Hodkinson 2009), diapause presumably occurs irrespective of environmental cues. Conversely, in multivoltine species, diapause decisions are likely to be under the control of external cues. For the few psyllids in which these cues have been examined, photoperiod is an important controlling factor (Wong and Madsen 1967; Nguyen 1975; Horton et al. 1998a; Mehrnejad and Copland 2005). Short days prompt diapause, whereas long days lead to continued development. Reproductive diapause of psyllids includes delays in both ovarian development and mating (Krysan and Higbee 1990; Horton et al. 1998a).

In this study, we examined whether development of potato psyllids under short-day conditions led to delays in maturation of ovaries and mating. Only limited field observations have been made for potato psyllid during winter months, so it was unclear to us whether this species would respond to short days by entering a reproductive diapause. In Washington, Oregon, and Idaho, adults of the Northwestern haplotype are readily found throughout the winter in association with *S. dulcamara* (Swisher et al. 2013c). In warmer regions (California), it has been suggested that all life stages of the Western haplotype can be found during the winter months (Essig 1917). These observations may indicate that response to photoperiod is less likely to occur in psyllids of the southern haplotypes than in psyllids of the Northwestern haplotype, as shown for

other Hemiptera that have extensive latitudinal distributions (Saulich and Musolin 2009). We tested whether response to photoperiod differed among psyllids from three widely separated geographic sources: south Texas (Central haplotype), California (Western haplotype), and Washington (Northwest haplotype). We additionally examined whether psyllids that had developed under short-day conditions differed in body size from psyllids that had developed under long-day conditions.

## MATERIALS AND METHODS

**Sources of insects.** Potato psyllids were collected from three geographic regions: south Texas, central California, and central Washington, U.S. Several hundred psyllids of the Central haplotype were collected in March 2013 from potatoes growing on the Texas A&M AgriLife Research farm near Weslaco, TX. Approximately 100 psyllids of the Western haplotype were obtained in February 2013 from potatoes growing in a greenhouse at the USDA-ARS station in Albany, CA. It is not known whether the psyllids were present in the greenhouse before the source plants had been placed in the greenhouse. Finally, several hundred adult psyllids of the Northwestern haplotype were collected on multiple dates between November 2012 and March 2013 from bittersweet nightshade (*S. dulcamara*) at locations near Wapato and Zillah, WA. Because of the geographic locations of the different source collections, we were almost completely certain of haplotype composition at the time of collection. Nonetheless, haplotype was confirmed in each collection by examination of mitochondrial DNA (mtDNA) sequences, using methods described by Swisher et al. (2013a). Subsamples of 12–20 reproductive females from each colony were examined. These subsamples were shown to support our conclusion that the psyllids used to initiate our three cultures were indeed each of the expected haplotype.

Psyllids were maintained in culture on potato plants (Russet Burbank) in BugDorm cages (61 × 61 × 61 cm; MegaView Science, Co., Taiwan). The three haplotypes were reared in separate greenhouses, each at a temperature varying between 15–27 °C. Cultures received continuous light, composed of ambient sunlight during daytime hours and

Other psyllid species (including other Trioziidae; Lauterer 1982) are known to exhibit seasonal polymorphisms in traits such as wing and body size (Hodkinson 2009), possibly associated with the onset of late-season dispersal tendencies (Hodgson and Mustafa 1984). Photoperiod has been shown to control seasonal polymorphism for the few species in which the trait has been examined experimentally (Wong and Madsen 1967; Nguyen 1985; Lauterer 1982).

supplemental lighting during the night hours (EnviroGro T5 fluorescent light fixtures, 4 bulb size; Hydrofarm, Petaluma, CA). The photoperiod trial was conducted from July to September 2013, following an estimated two to four generations in culture for each haplotype under the greenhouse conditions.

**Photoperiod trial.** We compared response of psyllids to two photoperiods: 10L:14D (hereafter, short day); and, 16L:8D (long day). The trial was done in two controlled environmental rooms, each set to a temperature of 22 (+1.1) °C. Temperature in each room was monitored using a Track-it Data Logger (Monarch Instrument, Amherst, NH). Lighting in each room consisted of two banks of EnviroGro T5 light fixtures. Psyllids were reared in cages (see below) placed directly beneath these lights. We used Russet Burbank potatoes grown from potato-seed pieces (Skone and Connors Produce, Warden, WA) throughout the trial. Single-eye seed pieces were removed from tubers using a 25 mm (diameter) melon baller, and planted singly in pots (9 × 9 × 8.5 cm). Plants were grown in a commercial potting soil (Miracle-Gro Moisture Control Potting Mix with Miracle-Gro Continuous Release Plant Food; Scotts Company, Marysville, OH).

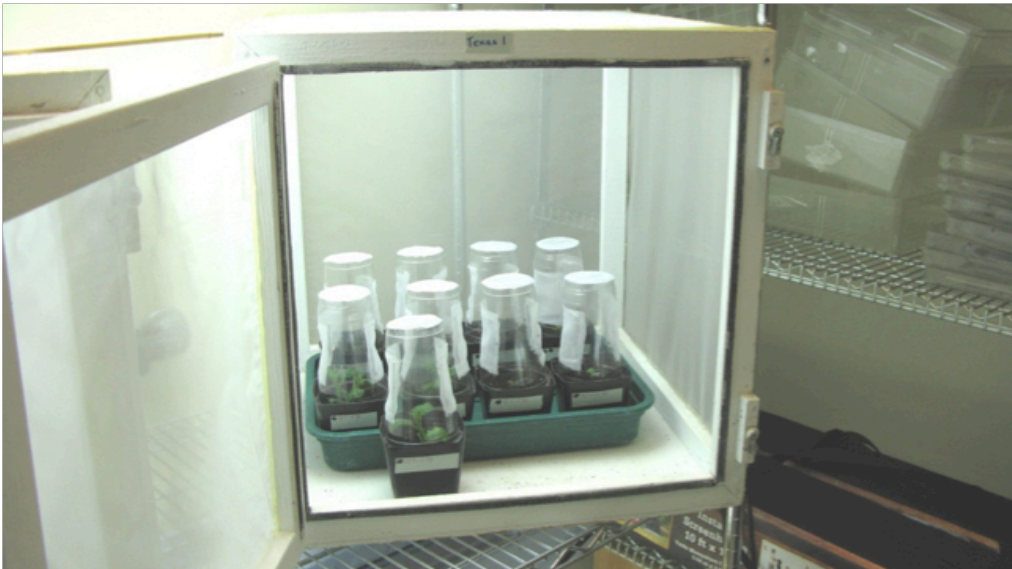
Three cages (56 × 51 × 60 cm) were placed in each room. Cages were constructed of organandy (tops and sides) and plywood. Weather stripping was used around the door of each cage to prevent psyllid escape. To ensure that any variation among psyllid haplotypes in response to photoperiod was due to treatment and not to a chance difference in quality of the rearing host, we designed the experiment to ensure that only one or two females were examined from a single rearing plant (i.e.,

rather than examining multiple females from a single rearing plant). We first placed six to nine newly emerged and psyllid-free potato plants into each cage in both photoperiod rooms. Approximately 20 psyllids of mixed sex of one haplotype were added to one cage in both rooms; the remaining two cages in both rooms received equivalent numbers of psyllids of the other two haplotypes. The psyllids were allowed to move freely among plants within a cage to oviposit. Plants were then monitored visually until fifth-instar nymphs were observed on the majority of plants within each cage.

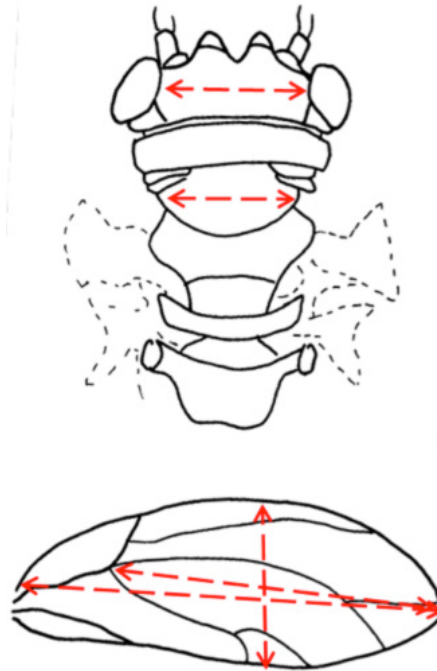
As fifth- (final-) instar nymphs became available on an individual plant, they were transferred using a small brush to a newly sprouted plant. We moved six final-instar nymphs of mixed sex (three males, three females) from a given natal plant onto a newly germinated plant, covered the newly infested plant with a ventilated plastic drinking cup (590 ml), and placed the newly infested plant into a new cage (Fig. 1), again according to haplotype. Nymphs were sexed by presence or absence of testes and appearance of the mycetome (Carter 1961). The original natal plant with any remaining nymphs was discarded. We repeated the transfer process for each of the six to nine original natal plants in a cage, thus producing six to nine newly

infested plants, each having six final-instar nymphs (Fig. 1). The inverted cups prevented movement by newly eclosed adults from a recipient plant onto a different plant within a cage. Covered plants were left undisturbed for 10 days to allow eclosion of adults. At 10 days, we randomly selected one or two females from each plant for measurement and dissection. The rearing of adults for dissection was initiated on three separate dates: 8 July 2013; 8 August 2013; and, 14 September 2013. Most of the dissected females were reared in the July and August periods (79.6% [125/157]) of dissected females). All three haplotypes were reared simultaneously at each photoperiod in all three rearings. By the end of the three rearing periods, sample sizes of dissected females were 22–29 psyllids per haplotype  $\times$  photoperiod combination, with each set of 22–29 females obtained from at least 20 different rearing plants.

Each female was first measured to determine wing size and body size. Five measurements were taken from the thorax, head, and wing for each female, and included two measures of wing length, and one measure each of maximum width of wing, distance between eyes (vertex), and width of the mesopraescutum (Fig. 2). A sixth measurement for each female was obtained by measuring the tibia from the right hind leg.



**Figure 1.** Cage and potato seedlings used in production of female potato psyllids for measurement and dissection.



**Figure 2.** Head, thorax, and wing measurements used for examining effects of photoperiod and haplotype on size of female psyllids. Measurements include three wing measures (two length, one width), distance between eyes (vertex), and width of mesopraescutum. Head and thorax, redrawn from Ossiannilsson (1992); wing, redrawn from Crawford (1911).

Measurements were obtained by first transferring a psyllid to a petri dish filled with alcohol and a layer of fine, white sand on the dish bottom. We used the layer of sand to position the psyllid for measurement of the vertex and mesopraescutum. Once those two measurements had been made, the right wing and right hind leg were removed and placed in three to four drops of alcohol on a microscope slide. The structures were oriented for measurement, covered with a cover slip to flatten them, and measured. All measurements

were made with a Leica MZ6 dissecting scope equipped with an ocular micrometer at 37.5x.

Once a female had been measured she was dissected to determine reproductive status. The psyllid was placed in six to seven drops of alcohol on a microscope slide. The insect was grasped by the posterior end of the abdomen and the base of the abdomen using two pairs of fine forceps, and carefully pulled apart. Generally, the spermatheca and ovaries would separate from the abdomen and be exposed for examination. If the organs were not



**Figure 3.** Reproductive classes exhibited by potato psyllids collected in November from *Solanum dulcamara* (Washington State): (A) immature ovaries, not mated; (B) immature ovaries, mated; and (C) mated with mature ovaries. Arrow points to sperm storage organ, filled with spermatophores.

sufficiently exposed, the abdomen was then carefully pulled apart along one side to expose the two organs. Females were categorized into one of three reproductive classes found by Horton (unpublished) to encompass the range of developmental types seen in November-collected adults of the Northwest haplotype (Fig. 3): (A) immature ovaries, not mated; (B) immature ovaries, mated; and, (C) mature ovaries, mated. Evidence that the female had been mated was provided by presence of spermatophores in the sperm-storage organ (Guédot et al. 2013). We additionally recorded for each female whether she contained fat, as diapause in other Hemiptera has been shown to be associated with an accumulation of fat (Horton et al. 1998b; Villavaso and Snodgrass 2004). Females showed varying quantities of fat upon dissection. We limited our scoring of fat levels to two categories: fat present (in any quantity); and, fat not visually apparent.

**Data analyses.** No statistical tests were done to examine effects of photoperiod and haplotype on ovarian maturation and mating

status, as essentially 100% of the psyllids were found to fall into a single developmental category (see Results). Presence or absence of fat in dissected females was examined using binary logistic regression with haplotype and photoperiod as explanatory variables. A main effects model was fitted and compared to the saturated model (main effects plus interaction) by calculating a chi-square deviance statistic (Allison 1999). The analysis was done using PROC LOGISTIC (SAS Institute 2010). Principal components analysis (PCA) was used to examine whether the six body measurements separated specimens by photoperiod or haplotype. The analyses were done in PROC PRINCOMP (SAS Institute 2010). Statistical separation of haplotypes or photoperiods was done using a  $3 \times 2$  (haplotype  $\times$  photoperiod) factorial analysis of variance conducted on the scores from the first and second principal components. The analyses were done using PROC GLM (SAS Institute 2010).

## RESULTS

Dissections showed that virtually all females had been mated and contained mature eggs, irrespective of photoperiod or haplotype (Table 1). Only three females showed either an absence of ovarian development or an absence of spermatophores (Table 1). We

failed to find spermatophores in two females having mature ovaries, which indicates either that females of this species may mature their ovaries even in the absence of mating, or that the spermathecae of these two females had become depleted following mating, as shown

**Table 1**

Numbers of potato psyllids of three haplotypes reared from egg to adult eclosion at either long- or short-day photoperiods falling into each of several reproductive classes (determined by dissection). Open cells (--) indicate no females exhibited that category of reproductive development.

	Photoperiod	No. females	Mature eggs present		No ovarian development	
			Mated	Unmated	Mated	Unmated
CA (Western)	Long	29	28	1?	--	--
	Short	29	29	--	--	--
WA (Northwestern)	Long	29	29	--	--	--
	Short	22	21	--	1	--
TX (Central)	Long	28	28	--	--	--
	Short	23	22	1?	--	--

<sup>a</sup> Potentially mated females having depleted spermathecae (Guédot et al. 2013)

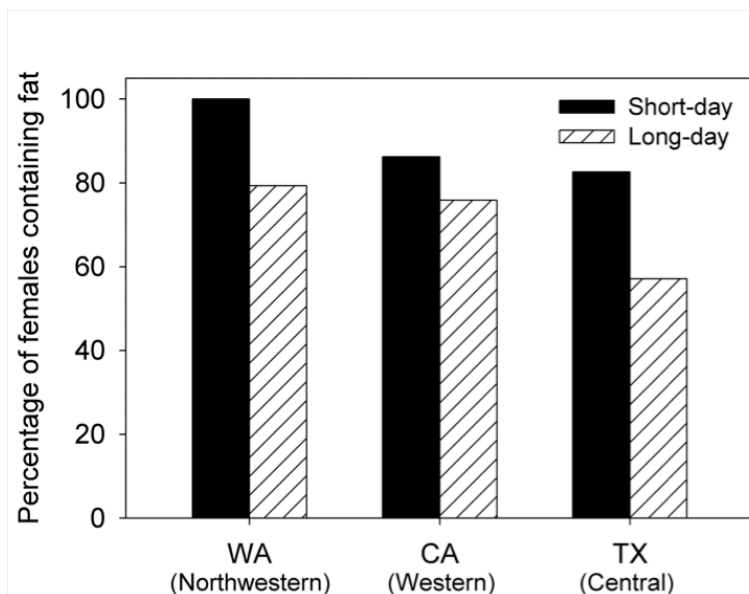
**Table 2**  
Eigenvectors for the first two principal component axes.

Body measure	Axis 1	Axis 2
Wing length (1)	0.46	-0.29
Wing length (2)	0.46	-0.30
Wing width	0.43	-0.11
Vertex	0.33	0.61
Mesopraescutum	0.33	0.63
Tibia	0.42	-0.23

by Guédot et al. 2013. A high percentage of females was found to contain some fat (Fig. 4), although that percentage was found to be affected by both photoperiod and haplotype. Binary logistic regression showed that short-day psyllids were statistically more likely to contain fat than long-day insects (Fig. 4:  $\chi^2 = 8.0$ ,  $df = 1$ ,  $P = 0.005$ ). There was also a marginally significant effect of haplotype on probability that a female contained fat ( $\chi^2 = 6.2$ ,  $df = 2$ ,  $P = 0.046$ ). The statistical effect was due to a significantly higher probability for the Washington psyllids than psyllids from southern Texas (Fig. 4:  $\chi^2 = 5.9$ ,  $df = 1$ ,  $P = 0.015$ ). Addition of the photoperiod  $\times$  haplotype interaction term to the main effects model did not significantly improve model fit (deviance = 3.4,  $df = 2$ ,  $P = 0.18$ ).

Two principal components were extracted explaining 70% (axis 1) and 15% (axis 2) of

the variation in measurements. All variables showed positive loadings along the first principal component (Table 2), suggesting that this component reflects variation in overall body size. The second axis appeared to describe variation in specimen breadth (Table 2: mesopraescutum and vertex). A biplot of component scores showed that psyllids clustered by haplotype, albeit with overlap among clusters (Fig. 5A; the scatter plot pools the two photoperiods). Mean haplotype scores are shown as stars horizontally along the top axis (PC1 means) or vertically along the right axis (PC2 means) of the scatter plot (Fig. 5A). Variables having loadings of 0.4 or higher (Table 2) were examined in detail. These variables included the three wing measures and tibia length, all of which were positively correlated with component scores along axis 1 (Fig. 5B–E). Correlation plots (Fig. 5B–E) and

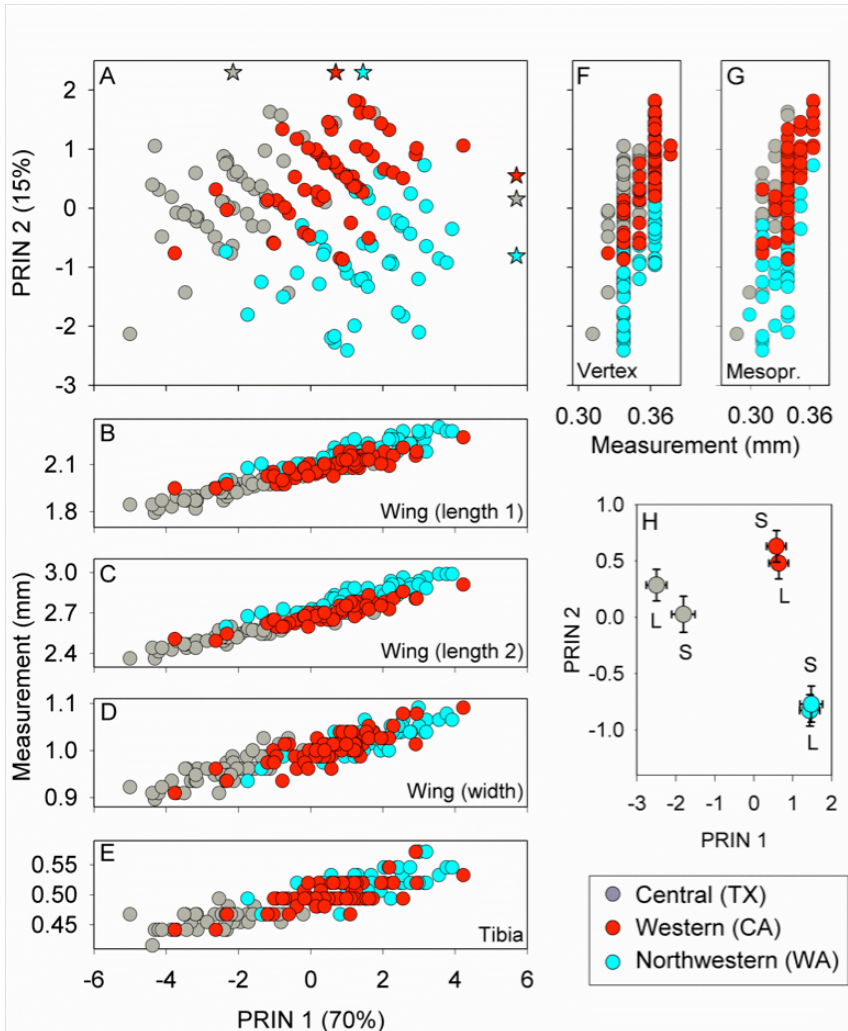


**Figure 4.** Percentage of dissected females containing some quantity of fat as function of haplotype and photoperiod.

mean PC1 scores (Fig. 5A) suggest that wings were longer and wider, and that the hind tibia was longer, in psyllids from Washington (Northwest haplotype) and California (Western haplotype) than in psyllids from Texas (Central haplotype). Variables showing large loadings along the second axis included the two body-width measurements (Fig. 5F–G). Axis 2 scores were positively correlated with the distance between the eyes (vertex)

and width of the mesopraescutum. The scatter plots suggest that psyllids from Washington State (Northwestern haplotype) were narrower than psyllids from the two southern locations.

Analysis of variance showed that mean component scores (Fig. 5H) varied with haplotype (PC1 scores:  $F_{2,151} = 92.1$ ,  $P < 0.0001$ ; PC2 scores:  $F_{2,151} = 45.3$ ,  $P < 0.0001$ ), but not with photoperiod (L vs S in Fig. 5H; PC1 scores:  $F_{1,151} = 1.0$ ,  $P = 0.33$ ; PC2 scores:



**Figure 5.** Results of principal components analysis. (A) Scatter plot showing clustering of haplotypes along first and second principal component axes; stars along top and right of plot are haplotype means. Photoperiods pooled. (B–E) Scatter plots showing correlation of four body measures with axis 1 scores, for each of the three haplotypes. (F–G) Scatter plots showing correlation of two body measures with axis 2 scores, for each of the three haplotypes. (H) Least squares means ( $\pm$  SEM) from factorial (haplotype  $\times$  photoperiod) analysis of variance for axis 1 scores and axis 2 scores. L: long-day females; S: short-day females.



$F_{1,151} = 0.1$ ,  $P = 0.82$ ); the interaction term was also non-significant for both PC1 and PC2 scores ( $P > 0.30$ ). Means separation tests were used to examine Tukey-adjusted differences between haplotype pairs. Those tests showed that mean PC1 scores differed among all paired comparisons (see means in Fig. 5A and Fig. 5H), with evidence that psyllids from Washington State were larger than psyllids from either Texas ( $P < 0.0001$ ) or California

( $P = 0.005$ ), and that psyllids from Texas were smaller than psyllids from California ( $P < 0.0001$ ). Tukey separation of mean scores along the second axis indicated that psyllids from Washington State were narrower than psyllids from either of the other two geographic sources ( $P < 0.0001$  for both comparisons), and that psyllids from Texas were on average narrower than those from California ( $P = 0.008$ ).

## DISCUSSION

The overwintering biology of potato psyllid is very poorly understood despite the efforts of entomologists beginning in the early 1900s to advance our understanding of this important life-history trait (Romney 1939; Wallis 1955; Pletsch 1947). The apparent predisposition of potato psyllid to disperse from southern “breeding” regions (Romney 1939) into northern growing regions of the central and western U.S. prompts questions about the overwintering fate of those dispersing psyllids, with no clear answers at this time. Not knowing the winter fate of these psyllids complicates pest-management decisions for northwest potato growers, as growers cannot anticipate with any degree of certainty when (seasonally) psyllids might arrive in their fields: i.e., primarily in summer as migrants from the south, or primarily in spring and early summer following local overwintering. The recent discovery that this pest species is a mix of at least four genetically distinct haplotypes (Swisher et al. 2012, 2014) further confuses the overwintering issue, as it is becoming apparent that genetic differences among psyllids can translate into biological differences (Liu and Trumble 2007). Those biological differences seemingly could include aspects of overwintering. We now have evidence that at least one haplotype (Northwestern) is present during the winter in Washington and Idaho, where it overwinters in association with a perennial nightshade, *Solanum dulcamara*, either in leaf litter or on the leafless stems of the plant (Jensen et al. 2012; Murphy et al. 2013; Swisher et al. 2013c). Those observations prompted the question of whether this northern haplotype might be more likely than the two southern

haplotypes to have a photoperiod-induced reproductive diapause.

The biological literature includes a wealth of observations for the Psylloidea about life-history traits associated with overwintering, including information on voltinism, importance of shelter plants for overwintering, and life-history stages known to overwinter (Conci et al. 1996; Lauterer 1999; Hodkinson 2009). There is, however, much less detail available about what environmental cues might control overwintering decisions by these insects (Hodkinson 2009). Unsurprisingly, the most detailed information is for highly damaging pest species, particularly the pear psyllids (*Cacopsylla* spp.). Both *Cacopsylla pyricola* (Förster) and *Cacopsylla pyri* (L.) have been shown to produce an overwintering phenotype in response to short-day conditions (Wong and Madsen 1967; Nguyen 1985; Horton et al. 1998a). Insects of either sex that have developed under short-day conditions are substantially larger than psyllids that have developed under long-day conditions (Wong and Madsen 1967; Nguyen 1985). Moreover, short-day females delay both mating and ovarian development (Krysan and Higbee 1990), even if they are subsequently moved to long-day conditions (Horton et al. 1998a). Mating and ovarian development by field populations of the overwintering generation in northern growing regions are delayed until temperatures begin to warm in late winter and early spring (Krysan and Higbee 1990; Horton et al. 1998a).

In this study, potato psyllids failed to exhibit a reproductive diapause in response to short-day conditions (Table 1). Virtually 100% of females of all three haplotypes mated and

matured ovaries under both long- and short-day conditions. Thus, we found no evidence to support our hypothesis that reproductive response to photoperiod among psyllids of the northern haplotype (Washington State) might be different than that among psyllids from the two southern haplotypes. Photoperiod also failed to have statistically significant effects on body size, indicating that there was no evidence for a photoperiod-induced size polymorphism for any of the haplotypes. There was a significant effect of photoperiod on percentage of females containing fat. Females that had been raised under short-day conditions were statistically more likely to contain at least some fat than females reared under long-day conditions (Fig. 4). We are unsure at this time about the possible biological significance of this result, although it is known for other Hemiptera that short-day insects accumulate fat as part of late-season preparation for overwintering (Horton et al. 1998b; Villavaso and Snodgrass 2004).

There was very strong statistical support for morphometric separation of the three haplotypes (Fig. 5H), and this result could be of eventual practical benefit. Currently, discriminating among haplotypes requires tedious and expensive examination of mtDNA sequences (Swisher et al. 2012). It would be useful to have a set of external diagnostics that could be used by extension personnel or pest-control advisors to discriminate among haplotypes as they monitor fields for presence of the psyllid, especially if we discover that haplotypes differ in how effectively they acquire and vector the bacterium that causes zebra chip disease in potatoes. Our morphometric results suggest that it may indeed be possible to discriminate

among haplotypes by use of external traits, although this idea will require confirmation by examination of field-collected insects.

Adult potato psyllids have been collected in midwinter from mats of *S. dulcamara* in Washington, Idaho, and Oregon (Murphy et al. 2013; Swisher et al. 2013c). Analysis of mtDNA indicated that virtually all of these overwintering psyllids were of the Northwestern haplotype, although scattered individuals of the Western haplotype were also shown to be present (Swisher et al. 2013c). Horton (unpublished data) has collected overwintering potato psyllid (likely of the Northwestern haplotype) that exhibit any of the three reproductive classes shown in Figure 3, suggesting that the psyllid was not overwintering at those locations in reproductive diapause. The photoperiod trials (Table 1), combined with these winter observations, prompt us to suggest that psyllids of the Northwestern haplotype overwinter in northern growing regions in a state of cold-induced quiescence, rather than in a true diapause. This same observation has been suggested for other Triozidae (Lauterer 2011), including other *Bactericera* (Lauterer 1982). Indeed, many Triozidae, including some populations of potato psyllid (Essig 1917), overwinter in a mixture of life-history stages (Lauterer 1991, 2011; Conci et al. 1996; Lauterer and Malenovský 2002; Hodkinson 2009), which also seems to be evidence for the absence of a photoperiod-induced diapause. Additional collection of potato psyllids from overwintering habitats throughout the species' range, accompanied by laboratory assays, would be useful to examine this hypothesis for all haplotypes of potato psyllid in multiple geographic regions.

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