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**COVER: *Agapostemon* sp. (Hymenoptera: Sphecidae)**

*Ammophila* sp. wasps on their nighttime perch, McDonald Beach, Richmond BC. July 24, 2014. These wasps spend the night in aggregations, clinging to vegetation with their mandibles.

**Photograph details:**

Photograph made by Sean McCann using a Canon 100mm macro lens and a Canon 60D. ISO 320;1/160th; single diffused flash above and to left with a bounce card.

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

DAVID R. HORTON<sup>1</sup>, EUGENE MILICZKY<sup>2</sup>, JOSEPH E. MUNYANEZA<sup>2</sup>, KYLIE D. SWISHER<sup>2</sup>, and ANDREW S. JENSEN<sup>3</sup>

## 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; Liefting 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; Liefting 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. 2013e). 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 Triozidae; 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.

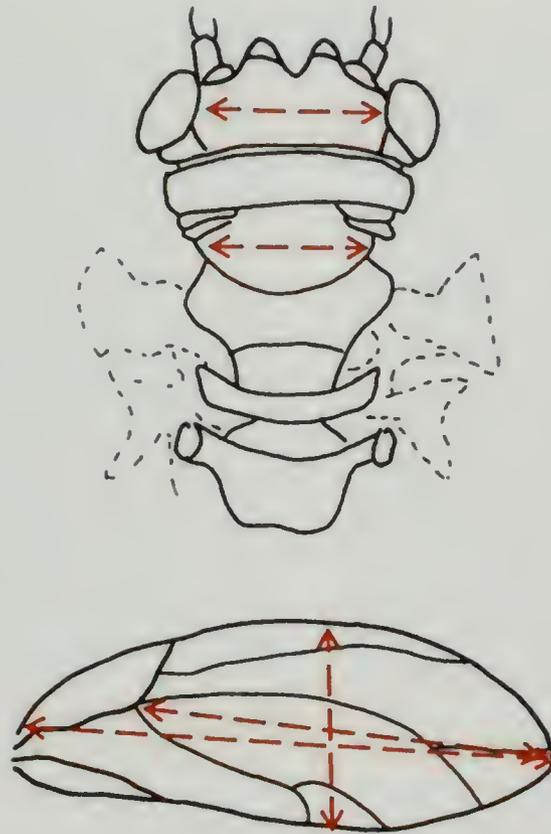
As fifth- (final-) instar nymphs became available on an individual plant, they were transferred using a small brush to a newly sprouted plant. We moved six final-instar nymphs of mixed sex (three males, three females) from a given natal plant onto a newly germinated plant, covered the newly infested 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**

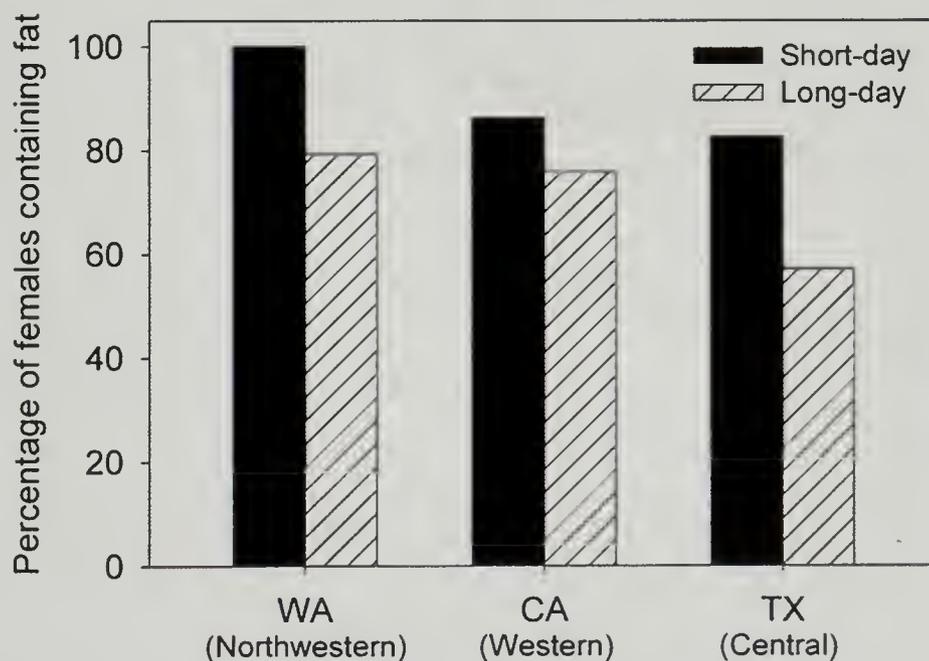
Eigenvectors for the first two principal component axes.

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

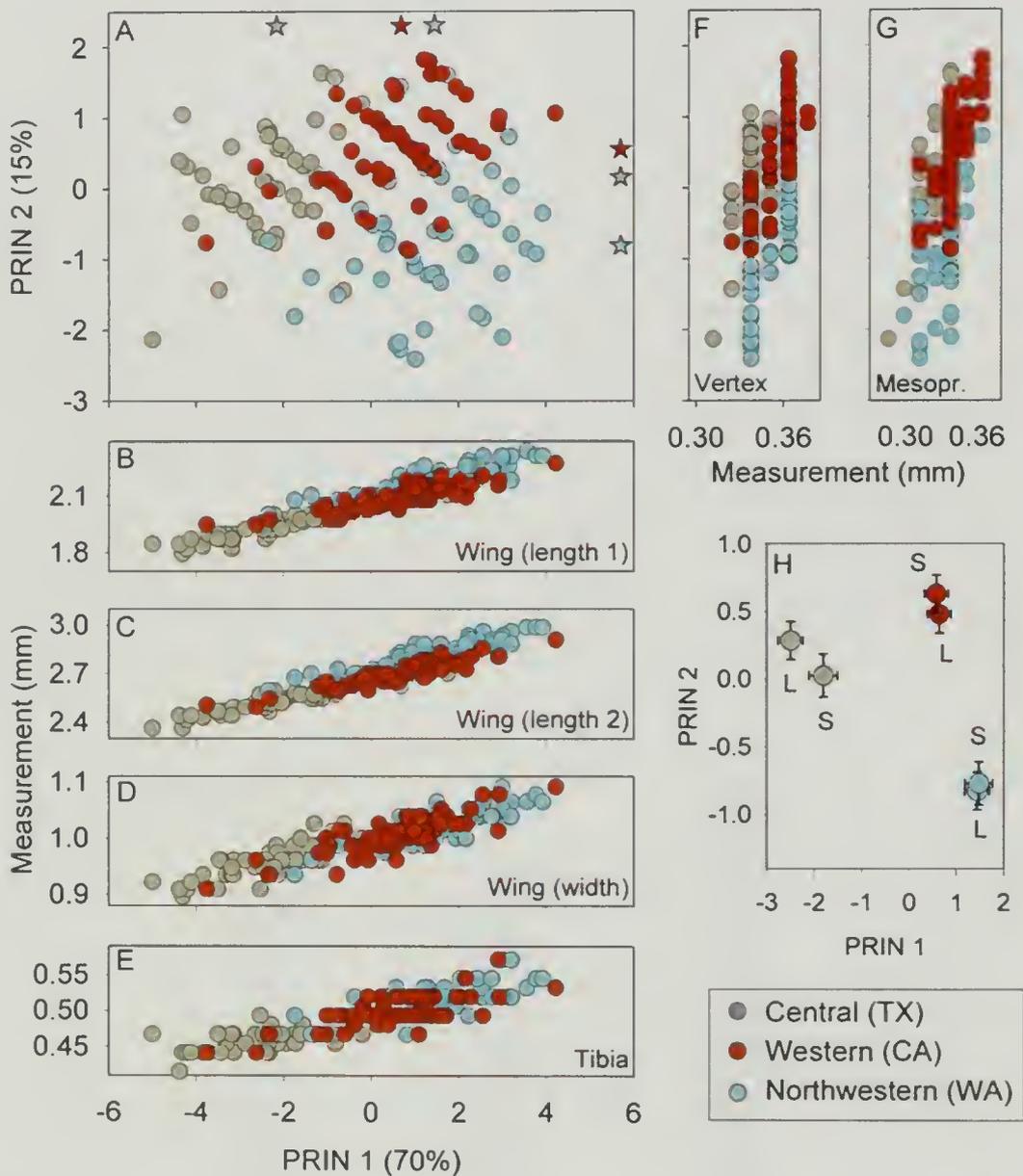


**Figure 4.** Percentage of dissected females containing some quantity of fat as function of haplotype and photoperiod.

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.

## ACKNOWLEDGEMENTS

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## SCIENTIFIC NOTE

**A new record of *Culex restuans* Theobald (Diptera: Culicidae) in British Columbia**SEAN MCCANN<sup>1</sup> and PETER BELTON<sup>1</sup>

*Culex restuans* is a New World mosquito species closely related to and resembling the cosmopolitan house mosquito *Culex pipiens* L. Typically, *Cx. restuans* lays its egg rafts on the surface of woodland pools, but it has adapted to lay its eggs in artificial containers. Immature stages can be found in water-filled containers from abandoned appliances to used tires that harbour algae. The immature stages of *Cx. restuans* can develop in water that ranges from clear to highly polluted. Like *Cx. pipiens*, the females obtain blood meals from birds, but can also feed on a broad range of mammal and reptile hosts (Hayes 1961). For that reason, the females have been identified every year since 2002 as a carrier and potential vector of West Nile virus (Ebel et al. 2005).

Its life cycle is similar to our other *Culex* species. Those adults that eclose in the late summer and fall mate, but the females are in reproductive diapause and feed only on carbohydrates before overwintering. In rural habitats, they probably search out underground burrows and similar sites to hibernate, but in anthropic habitats, they are common in basements and outbuildings.

Its known distribution in America ranges from the eastern half of Alberta south and east to Guatemala, and it is common in southeastern Canada and the eastern and central United States. There are fewer records in the west, although according to Darsie and Ward's (2005) authoritative work, the insect's range extends to the west coast through Wyoming, southern Idaho and Oregon. Well-substantiated records in southwestern California exist.

We describe the first verifiable record of this species in British Columbia (B.C.) from a photograph taken by one of us (McCann) in Goldstream Provincial Park, on Vancouver Island, about 16 km northwest of Victoria, on 10 October 2010 (Fig.1). The *Cx. restuans*

female was found under a bridge deck alongside *Cx. tarsalis* and *Culiseta incidens* females. Its pale abdomen was distended with hypertrophied fat body and it was probably ready to hibernate but unfortunately escaped capture. The paired patches of pale scales on the scutum, which were clearly visible, allow reliable differentiation of this individual from *Cx. pipiens*. The scutum of *Cx. pipiens* has never been reported with the pale spots or the pale scales that are clearly visible above the wings on the scutum of the female we photographed, confirming our identification.

Three other records of this species in or close to B.C. have been explained as misidentifications or accidental introductions. The provincial record is of a female taken in a light-trap at Esquimalt (Twinn 1945). The specimen is lost, and Wood et al. (1979) considered it to be a misidentified *Cx. pipiens*. Further east, there are records of *Cx. restuans* near the northern border of the Idaho panhandle, just south of Creston, B.C. (State of Idaho 1985). There seem to be no specimens confirming these records. Darsie and Ward include their localities on the distribution map of the first edition of their monograph (Darsie and Ward 1981), but not in the second (Darsie and Ward 2005, Plate 29B). The third record is from Island County, in northwestern Washington, close to Seattle and Tacoma. Sames et al. (2007) point out that this record is "far outside the distribution range"—depicted in Darsie and Ward (2005)—and suggest that "this species may have been accidentally introduced into Island County" by tourists or from nearby ports.

We believe our specimen lends credence to Twinn's 1945 identification, because Esquimalt is within flying range (9 km) of Goldstream Provincial Park, and both are just across the Salish Sea (63 km) from Island County, WA. We suspect that these western records are from established populations that

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**Figure 1.** *Culex restuans* female; photograph by S. McCann. Note two patches of pale scales on scutum (inset, arrows) that identify this as *Culex restuans*, and the distended abdomen typical of overwintering *Culex* females.

have simply not been noticed. The recent interest in West Nile virus vectors has focused on identifying females, but many of those captured in light traps have the scales on their scuta denuded. Even unrubbed specimens may not show the pale scale patches. Fortunately,

the males and larvae of *Cx. restuans* are quite distinct from those of *Cx. pipiens*, and we continue to search for them and urge collectors to look closely at specimens taken near these locations and elsewhere in B.C. and its neighbouring states.

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## OBITUARY

**Michael James Smirle (1954-2014)**GARY JUDD<sup>1</sup>

Dr. Michael J. Smirle passed away on July 27, 2014, at his home in Summerland, British Columbia (B.C.), while working in what he affectionately referred to as his Mother's garden. Born in Abbotsford, B.C., on June 18, 1954, Mike grew up on the family farm in the nearby village of Bradner. He was the only child of Jim and Joan Smirle.

While growing up, he excelled at piano, academics and basketball. After graduating top all-round student from Abbotsford Senior Secondary in 1972, Mike began his academic career in the faculty of music at the University of British Columbia (UBC). When his father passed away in January, 1974, Mike took the year to travel extensively with his mother. He returned to UBC in the fall of 1974, this time enrolled in the Faculty of Science.

It was at UBC, on October 5, 1974, that Mike met Mary-Anne, his wife to be, and they began their journey in life together. He graduated in 1979, with a Bachelor of Science in Biochemistry, after which he returned to his daffodil farm in Bradner, and worked part time as a lumber grader in the local mill.

Tiring of shift work, Mike returned to university, this time at Simon Fraser University (SFU), where he pursued a Masters in Pest Management. While studying under Dr. Mark Winston, Mike combined an interest in biochemistry with a love for honey bees, which he had cultivated as a child on the family farm. His Master's Thesis "Development of a Sensitive Bioassay for Evaluating Sub-lethal Pesticide Effects on the Honey Bee" was followed by doctoral studies, also at SFU, on the "Insecticide Resistance Mechanisms in the Honey Bee". His doctoral studies earned him a prestigious J.H. Comstock Award from the Pacific Branch of the Entomological Society of America.

Upon completion of his Ph.D. in 1989, Mike worked as a sessional instructor at SFU, while undertaking a post-doctoral research fellowship at UBC with Dr. Murray Isman.

Mike's post-doctoral research focused on the metabolism of secondary plant compounds in phytophagous insects. He examined the mode of action of natural-product insecticides, especially neem oil and other allelochemicals.

While working two years in Isman's laboratory, Mike published three scientific papers and a significant book chapter on Allomones in *Insect-Plant Interactions*. More importantly, he also became close friends with Dr. Tom Lowery, with whom he collaborated for the rest of his career.

On the February 1, 1990, work brought Mike and the family, which now included sons James and Jeffery, to their home in Summerland, B.C. Mike worked at the Pacific Agri-Food Research Centre (PARC) as a respected and valued research scientist until his passing. Working at PARC was like coming home, as Mike shared his career with no less than seven Masters in Pest Management graduates from SFU, as well as Tom Lowery from UBC. During his 24-plus years at PARC, Mike led a successful research team that focused on insect toxicology and determined the biochemical mechanisms of insecticide resistance—knowledge essential for developing strategies to manage resistant pest populations. With his capable technician Cheryl Zurowski always in support, he contributed to the development of pest management systems for orchard and grape crops, as well as to our understanding of the environmental fate of new insecticides. His collaboration on the Canadian International Development Agency Hebei Dry-Land Farming Project led to his becoming an Adjunct Professor at the University of Beijing, China.

Mike published 34 papers in peer-reviewed journals, two book chapters and 58 miscellaneous technical articles throughout his career. At PARC, he served as Head of the Environmental Studies Section for a number of years, eventually becoming the Test Site

<sup>1</sup> Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B.C.

Manager for the Minor Use Pesticide Program at Summerland. He was a longtime member of the Entomological Societies of Canada (ESC) and British Columbia (ESBC). He served as an Associate Editor to the ESC (2006–2014) and was on the Editorial Board of Biopesticides International (2004–2104). He was the President of the ESBC at the time of his passing.

Outside of work, Mike's greatest joy was spending time with his boys and their friends. He loved every moment spent on the sidelines of local soccer pitches and track and field venues, attending his sons' high school plays, or, most especially, cheering on James and Jeff at the hockey rink—watching them thrive and

grow into the incredible young men they are today. Mike's lifelong love of music allowed him to share his talents as a regular organist at Holy Child Parish, and singing with the Mosaic Vocal Ensemble was a favorite pastime during his years in Summerland.

It always comes as a shock when the entomological community loses one of its valued scientists. This tragedy is compounded when that scientist has also been a great human being and a close friend for over 30 years.

Mike was all of these things.

He will be dearly missed by everyone fortunate enough to have known him.

## Presentation Abstracts

### Entomological Society of British Columbia Annual General Meeting, Simon Fraser University, Burnaby, B.C., Oct. 24, 2014

#### **Predators on the farm: Augmentative releases for biological control of blueberry aphid in British Columbia**

*R. McGregor, A. Martins and K. Crisp, Institute of Urban Ecology, Douglas College, New Westminster, B.C.*

The practice of augmentative biological control is supported globally by a well-established industry that produces over 230 products. Despite this, adoption of augmentative releases for management of pests of field crops has been rare compared to the use of these products in greenhouse agriculture. Here, we describe the results of releases of *Micromus variegatus* (Neuroptera: Hemerobiidae) and *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) for management of the blueberry aphid (*Ericaphis fimbriata*, Hemiptera: Aphididae) on blueberries in British Columbia (BC). *Ericaphis fimbriata* is the vector of blueberry scorch virus, a pathogen that causes serious economic damage to blueberry plantings in the province. Releases of both predators were made at three small, organic farms in June and July, 2014. Aphid populations at the three farms declined dramatically after releases approximately two to three weeks earlier than typical seasonal declines of aphids. Eggs of *A. aphidimyza* were observed in post-release samples at all three release locations, providing evidence that this predator reproduced. Augmentative releases of these biological control products clearly have a strong potential as a strategy for managing blueberry aphid, especially in organic plantings.

#### **Metabarcoding as a tool for assessing stream biodiversity and ecosystem function at pipeline crossings**

*M. Schwarzfeld, A.-M. Flores, A. Thielman, A. O'Dell, A. Costello, D. Erasmus, B. Murray, L. Poirier, J. Robert, M. Gillingham, J. M. Shrimpton and D. Huber, Natural Resources and Environmental Studies, University of*

*Northern British Columbia, Prince George, B.C.*

This study is designed as a before–after, control–impact (BACI) analysis of stream biodiversity and ecological processes at pipeline crossings. We are using a combination of morphological methods, DNA barcoding, and metabarcoding techniques to assess benthic invertebrate communities, environmental DNA in water, and aquatic food webs.

#### **Terrestrial invertebrate indicators of ecological function in alpine ecosystems**

*A. Thielman, D. Huber and M. Gillingham, Natural Resources and Environmental Studies, University of Northern British Columbia, Prince George, B.C.*

An invertebrate biodiversity monitoring and assessment program in northwest British Columbia is currently investigating ecological function in alpine ecosystems throughout construction of a natural gas pipeline and subsequent restoration activities. Preliminary 2014 results and a discussion of taxa collected and their potential for use as indicators of ecosystem function were presented.

#### **Impact of two contrasting marine habitats on decomposition and arthropod colonization of cadaver models in the Salish Sea**

*G. Anderson and L. Bell, Centre for Forensic Research, School of Criminology, Simon Fraser University, Burnaby, B.C.*

Pig carcasses were deployed on the ocean floor, in Saanich Inlet at 92 m, and in the Strait of Georgia at 300 m. Both sets of carcasses were deployed under a tripod that carried a high-definition video camera, oxygen optode and a seawater conductivity, temperature and density instrument package (CTD). Dramatic differences in colonization were noted.

**Is aggregated oviposition by blowflies *Lucilia sericata* and *Phormia regina* (Diptera: Calliphoridae) pheromone mediated?**

*W. Wong<sup>1</sup>, B. Brodiel, S. VanLaerhoven<sup>2</sup> and G. Gries<sup>1</sup>, <sup>1</sup>Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., and <sup>2</sup>University of Windsor, Windsor, Ontario*

Aggregative oviposition in female blowflies has been perceived as pheromone mediated. Our study with two species of blowflies demonstrated that the presence of female blow flies at varying reproductive stages on an oviposition site enhance its attractiveness to fellow female blow flies by depositing semiochemicals associated with feeding.

**Baculovirus and co-infection dynamics in an insect host, *Trichoplusia ni***

*J. Scholefield and J. Cory, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.*

When multiple pathogens co-infect a host, the outcomes can be far different from that of a single infection. We exposed *Trichoplusia ni* to a baculovirus, followed by a second pathogen. The presence of a competitor reduced the infection success of the baculovirus and had negative consequences for virus replication.

**Do short-term changes in atmospheric pressure affect the calling behavior of male crickets?**

*J. Pol<sup>1,2</sup>, A. Gould<sup>2</sup>, C. Guglielmo<sup>2</sup> and J. McNeil<sup>2</sup>, <sup>1</sup>Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., and <sup>2</sup>Department of Biology, Western University, London, Ontario*

Olfactory-based, pheromone-mediated mating behavior of species from different insect orders has been shown to be affected by short-term changes in atmospheric conditions. We examined the acoustic chirping behavior of male crickets to determine if similar behavioral changes occur and if the response to this environmental cue varies with age.

**Temperature effects on pea aphid (*Acyrtosiphon pisum*) "personalities" in the context of anti-predator behavioural**

**syndromes**

*D. Quach, F. Simon and B. Roitberg, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.*

There is a growing body of evidence for the existence of "personalities" within animals. We demonstrate that genetically identical individuals—pea aphids—can have personalities and change their predatory escape response across temperature, and examine whether the distribution of variation between individuals of the same clone was retained across these environments.

**Competitive behavioural strategies of *Drosophila suzukii* and *Drosophila melanogaster***

*T. Dancau and T. Stemberger, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.*

We investigated the adult competition dynamic between *Drosophila melanogaster* and *D. suzukii* females for a single resource. Resource-holding potential based on size as well as aggressive behaviours of varying intensities and costs were used to explore competitive behavioural strategies. Our results illuminate the complicated nature of aggressive interspecies interactions.

**Relative desiccation resistance of *Rhagoletis sister* species**

*J. Hill, K. Hausken, N. Shaffer and D. Schwarz, Department of Biology, Western Washington University, Bellingham, WA*

The invasive apple maggot fly is largely absent from the arid regions of the Pacific Northwest, which is occupied by its native sibling, the snowberry fly. We compare the desiccation resistance of both species and discuss potential consequences of interspecific hybridization on drought adaptation in the apple maggot.

**Red-throated Caracaras versus army ants as wasp predators in Neotropical forests**

*S. McCann, T. Jones, O. Moeri, C. Scott, S. O'Donnell and G. Gries, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.*

Predators of Neotropical social wasps have not been well studied, but historically, army

ants have been considered the most important wasp predators. We studied Red-throated Caracara provisioning behaviour. Our quantitative results suggest that these specialist predators may rival army ants as predators on social wasps.

### **When barcoding fails: Molecular identification of apple maggot flies in the face of gene flow**

*D. Schwarz, Department of Biology, Western Washington University, Bellingham, WA*

Economically important apple maggot flies and their non-pest snowberry maggot sibling species share too much genetic variation to be distinguished by a single gene. Instead, a modest number of unlinked single nucleotide polymorphisms provide a fast and cost-effective method to identify apple maggots despite ongoing introgression from snowberry maggots.

### **Apple Maggot (*Rhagoletis pomonella* Walsh): Resident quarantine pest in southwest British Columbia (Lower Mainland)**

*S. Glasgow and T. Hueppelsheuser, Plant Health Unit, Plant and Animal Health Branch, British Columbia Ministry of Agriculture, Abbotsford, B.C.*

A quarantine pest of apples, Apple Maggot (*Rhagoletis pomonella* W.) was first detected in British Columbia along the Canada-U.S border of the Lower Mainland in 2006. In this presentation we share the methods used and data collected during 2013/2014 monitoring of adult flight and fruit damage. Adult flight monitoring has given insight to the lifecycle of Apple Maggot in southern B.C., and we hope this is useful to commercial growers for timing their pest management activities. This project was funded in part by the BC Ministry of Agriculture and Agriculture and Agri-Food Canada through Growing Forward 2, a federal-provincial-territorial initiative.

### **Stark sexual display divergence among jumping spider populations in the face of gene flow**

*G. Blackburn and W. Maddison, Department of Zoology, University of British Columbia, Vancouver, B.C.*

Can selection prompt population divergence in the face of gene flow? If so, what traits are involved? We present genetic evidence that, in *Habronattus americanus* jumping spiders, selection can promote stark sexual display divergence in the face of gene flow even among closely related populations.

### **Genetic and ecological consequences of sex ratio distortion in a booklouse**

*C. Hodson and S. Perlman, Department of Biology, University of Victoria, Victoria, B.C.*

In a newly discovered species of booklouse, we have found that two female types exist: one that produces both female and male offspring and the other that produces only females. As this difference is stable across generations, it has population consequences. We discuss the genetic consequences, in terms of mitochondrial structure, and the ecological consequences, in terms of differences in reproductive potential between the female types.

### **The immune repertoire of a divergent, symbiont-defended *Drosophila***

*M. Hanson, F. Hamilton and S. Perlman, Department of Biology, University of Victoria, Victoria, B.C.*

*Drosophila melanogaster* is a wonderful model for studying the innate immune system. However, immunity in divergent lineages of *Drosophila*, with distinct ecologies and natural enemies, is largely unstudied. Using transcriptomics, we describe the immune pathways of the mushroom-feeding *D. neotestacea*, a natural system where hosts are commonly infected with a *Spiroplasma* defensive symbiont that protects against a virulent nematode parasite. Although the major immune cascades are well conserved, some antimicrobial peptides were absent from the *D. neotestacea* transcriptome.

### **What women want: Does bait influence the age of female *Drosophila suzukii* (Diptera: Drosophilidae) caught in traps?**

*K. Sim and T. Hueppelsheuser, Plant Health Unit, Plant and Animal Health Branch, British Columbia Ministry of Agriculture, Abbotsford, B.C.*

Bait preference is of major concern for effective monitoring of the crop pest

*Drosophila suzukii* Matsumura (Diptera: Drosophilidae), spotted wing drosophila (SWD). Catching SWD when it first becomes active in the spring has been a focal point for many bait studies; however, in areas where SWD populations are not completely inactive during winter months, such as south western B.C., it is difficult to pinpoint when SWD populations become an active risk to crops. Monitoring in these areas, in order to allow timely management decisions by commercial fruit growers, may benefit from tracking the presence of reproductively viable females in the population. To implement effective population age structure monitoring, it is important to determine if bait preference is influenced by female age. Here we compare the age classes of caught female SWD in two baits types, yeast solution and apple cider vinegar, to determine if age-based bait preference exists. This project was funded in part by the BC Ministry of Agriculture and Agriculture and Agri-Food Canada through Growing Forward 2, a federal-provincial-territorial initiative.

**How the false widow spider finds true love: Female contact pheromone elicits male courtship behaviour in *Steatoda grossa***

C. Gerak, C. Scott, S. McCann and G. Gries, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.

We investigated courtship behaviour and pheromonal communication in the false black widow spider, *Steatoda grossa*. Males engage in a complex pre-copulatory courtship ritual that includes dismantling the female's web (web-reduction behaviour). We show that a pheromone on the silk of virgin but not mated females elicits courtship behaviour by males.

**Temperature-induced changes in western tent caterpillar feeding behaviour as a mechanism for variability in NPV susceptibility**

P. MacDonald<sup>1</sup>, J. Cory<sup>1</sup> and J. Myers<sup>2</sup>, <sup>1</sup>Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., and <sup>2</sup>Department of Zoology, University of British Columbia, Vancouver, B.C.

Western tent caterpillar populations display cyclical dynamics with 8- to 11-year periodicity in southwestern B.C. Long-term

data shows that Nucleopolyhedrovirus (NPV) is an integral component of these dynamics, but climate might also be involved. We investigate how temperature influences individual feeding behaviour in western tent caterpillars to propose how climate might alter infection dynamics.

**Keeping up with climate change: Temperature and humidity effects on an insect herbivore in apple orchards**

J. Swain<sup>1</sup>, G. Judd<sup>2</sup> and J. Cory<sup>1</sup>, <sup>1</sup>Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., and <sup>2</sup>Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B.C.

We investigate how temperature and humidity contribute to the outbreak potential of *Spilonota ocellana* during seasonally vulnerable life stages. Our results indicate a threshold tolerance of spring larvae to sub-zero temperatures, such as those experienced during frost events, and optimum conditions for egg hatch and development.

**"Weeding" out climbing cutworm: The toxic, yet alluring power of shepherd's purse in vineyards**

N. DeLury, T. Lowery, K. Deglow and A. Mostafa, Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B.C.

We investigate the presence of shepherd's purse, *Capsella bursa-pastoris* (L.) (Brassicaceae), in vineyards as a potential means to control native and invasive climbing cutworm species—all serious pests of grape buds in south-central B.C.—with varying results.

# Symposium Abstracts: Dangerous Creatures? Arthropods Affecting Human Health—Fact and Fiction

Entomological Society of British Columbia  
Annual General Meeting,  
Simon Fraser University, Burnaby, B.C., October 25, 2014

Note: There was a total of seven papers presented in this symposium. We were able to obtain abstracts from six of the authors.

## **Bloodfeeding in Anophelines: context, context, context**

*B. Roitberg, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.*

I argue that bloodfeeding by Anopheline mosquitoes is best understood from a contextual perspective. Here, the contexts are: body size; body mass; and, energy state. First, I review state-dependent theory as tool for explicating context, and then I describe three sets of experiments that explore my contention. These experiments consider three different points in the bloodfeeding sequence: attempting to access a host that is protected by a bednet; feeding at a host that attempts to defend itself; and, post-feeding dynamics after feeding on healthy and plasmodium-infected hosts. In all three cases, the complex response by female Anophelines is best understood by applying multi-factorial models that include interaction terms. In other words, size effects cannot be understood by evaluating energy state, etc. This is context. I conclude by showing that these contextual responses by female mosquitoes can impact mosquito-vectored diseases such as malaria. My final point is that simplistic approaches that treat mosquitoes as if they were flying syringes are problematic and will have short shelf life.

## **West Nile virus in British Columbia**

*A. Furnell, British Columbia Centre for Disease Control, Vancouver, B.C.*

The British Columbia Centre for Disease Control is involved with West Nile virus because it is a reportable communicable disease. Our surveillance includes the mosquito component of the enzootic cycle. We use miniature Centre for Disease Control-issued light traps, baited with carbon dioxide, for field surveillance. Four *Culex* species exist

in British Columbia (B.C.); we collected three in our surveillance. *Culex pipiens* is widely distributed across the province and is especially abundant in urban centres, which have well-developed storm-sewer catch-basin systems, where *C. pipiens* young develop. This species was implicated as the primary vector in the initial North American outbreak. *Culex tarsalis* can be found in northern B.C., but they are mostly found in the south, along the Canadian border, where specimens infected with West Nile virus were detected in the Okanagan Valley. *Culex territans* is commonly found in ponds or artificial containers that hold water, but are seldom collected in our light trap surveillance. They feed on amphibians, so are not considered part of the West Nile virus enzootic cycle. There is a new record of *Culex restuans* being identified on Vancouver Island. This common vector is associated with virus amplification in birds in the prairie provinces and in eastern Canada, but it is rare for B.C.

## **Innate immune responses of vectors to pathogens: What defines vector-pathogen specificity?**

*C. Lowenberger, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.*

Certain vectors transmit specific pathogens that may cause disease in humans or other animals. Why do vectors transmit a limited number or type of pathogen/parasite and kill others? The innate immune system of insects, including vectors, evolved to recognize and eliminate microbial pathogens. The pathogens in turn have evolved to avoid, evade, or inactivate components of the immune system for their own benefit. Plasmodium, the causal agent of human malaria, develops in an area of the midgut that has almost no expression of

anti-parasite peptides. The parasite *Trypanosoma cruzi*, which causes human Chagas disease, never leaves the gastrointestinal (GI) tract of its kissing bug vector, but is transmitted through fecal transmission as it feeds on blood. If this parasite leaves the GI tract to enter the body cavity, it is killed by immune molecules. Human dengue virus, transmitted by *Aedes aegypti*, is often killed by components of the apoptotic pathway: the virus enters cells and is recognized, and the cells initiate apoptosis. However, the virus induces the expression of inhibitors of apoptosis (IAPs) that delay apoptosis until the virus has replicated. The interplay between the innate immune system and specific parasites and pathogens determines which vectors transmit which pathogens to humans.

#### **Ticks: The perspective of a physiologist**

*R. Kaufman, Professor Emeritus, University of Alberta, Salt Spring Island, B.C.*

Whenever I have occasion to mention that my research area at the University was the physiology of ticks (and still is here on Salt Spring Island), 99.99% of the time the response is something like, "Oh, do you work on Lyme Disease?" Actually, I don't, but the response obviously reflects the general view that the only reason to do research on a nasty creature like a tick is to find out ways to eliminate them and the diseases they transmit! Fair enough, but I like to emphasize that even invertebrates, including ticks, can teach us a lot about physiological mechanisms in general. In this presentation I'll tell you that female ticks of the family Ixodidae regulate their body fluid composition while feeding on the host by secreting an enormous volume of saliva back into the host's circulation; this is how pathogens from infected ticks enter the host. I outline the control mechanisms of salivation: Basically, an interaction exists among several neural pathways that involve the neurotransmitters acetylcholine, dopamine, and  $\gamma$ -aminobutyric acid (GABA), and a pathway in which some ergot alkaloids are mimics. In most ixodid ticks, copulation must occur on the host, after both sexes have fed at least partially. After engorgement, the salivary glands degenerate by an autolytic process that is triggered by an ecdysteroid hormone and modified by a so-called "male factor". This

male factor serves more importantly as an "engorgement factor" because, without it, virgin females feed to only about one-tenth the normal engorged weight. The engorgement factor consists of two proteins ( $\alpha$ - and  $\beta$ -voraxin). When we immunized a rabbit against the two proteins, 75% of the normal mated females that fed on it failed to feed beyond the small size characteristic of virgins. This has implications for developing a biological control mechanism against ticks and the pathogens they transmit.

#### **Natural products to control bed bugs**

*Y. Akhtar and M. B. Isman, Faculty of Land and Food Systems, University of British Columbia, Vancouver, B.C.*

In recent years, infestations by bed bugs, *Cimex lectularius* L., (Hemiptera: Cimicidae), have increased dramatically in many parts of the world, including Canada and U.S.A. This has led to renewed interest in the development of products of dubious composition and efficacy to control the infestations. The exact cause of the resurgence of bed bugs is unclear, but may be a consequence of the development of resistance in the insects to commonly used synthetic insecticides, including pyrethroids, along with other factors. Although there is no evidence that bed bugs transmit disease between human hosts, they cause a range of emotional problems and have an especially negative impact on the hospitality industry. Urgent need exists to develop pest management tools that are effective in suppressing bed bug populations and do not themselves have undue negative impacts on human health. We discuss semiochemicals and diatomaceous earth (DE) as part of a bed bug pest management system. Approximately 150 compounds (provided by SemiosBio Technologies Inc.), including both natural and synthetic semiochemical analogs, were screened, using glass arenas, for repellent effects. Some of the compounds demonstrated sufficient bioactivity against bed bugs to be considered for continued repellent formulation development. Similarly, different samples of DE, provided by DE Labs Inc., were screened for toxic effects against *C. lectularius*. Based on LC50 values, DE Labs Inc. and Mother Earth DE-dusts were significantly more toxic than other DE-dusts. The use of these products

may represent a new, low-impact standard for public health pest control.

**The truth about spider bites: “Aggressive” spiders and the threat to public health**

*Catherine Scott, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.*

Current sensational media coverage indicates widespread ignorance about the basic biology of spiders and other arthropods. I argue that spiders should not generally be considered dangerous, but that misconceptions and misdiagnoses pose a greater threat to public health. There are only two genera of medically significant spiders in North America: *Latrodectus* (black widows) and *Loxosceles* (recluse spiders). Only the former is found in Canada. Spiders are generally unaggressive and bite only defensively. In places where widow and recluse spiders are common, bites are very rare. Physicians seem quick to blame unexplained bites or lesions on

spiders in the absence of any evidence. Many other arthropods depend on blood meals from human or other vertebrate hosts and are thus more likely culprits for mysterious bites. Bacterial infections and a host of other conditions are commonly misdiagnosed as ‘necrotic arachnidism’, and lack of proper treatment can lead to complications and even death. Most spider bites are benign and do not require medical intervention. Spiders are far more useful than harmful as voracious predators of household and agricultural pests. I am optimistic that, through education and outreach, we can inspire people to respect and learn from these beautiful and amazing arthropods.

**Tick distribution and tick-borne diseases in British Columbia**

*Muhammad Morshed, BC Centre for Disease Control*

No abstract submitted







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