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# Haplotypes of the Potato Psyllid, *Bactericera cockerelli*, on the Wild Host Plant, *Solanum dulcamara*, in the Pacific Northwestern United States

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**Abstract** *'Candidatus* Liberibacter solanacearum' (Lso) is a bacterium that infects solanaceous crops and causes plant decline and yield losses, especially in potato and tomato. Lso is transmitted to these hosts by the potato psyllid (*Bactericera cockerelli* Sulc) vector. *B. cockerelli* host plants are not limited to crop plants, but also include many wild, solanaceous weeds. These wild hosts could potentially impact overwintering and breeding of the psyllids and serve as reservoirs for Lso. In the Pacific Northwestern United States, *B. cockerelli* was recently reported to overwinter on bittersweet nightshade (*Solanum dulcamara* L.). The present study utilized high resolution melting analysis of the *B. cockerelli* mitochondrial cytochrome *c* oxidase I gene to assess the psyllid populations occurring on *S. dulcamara* during the summer and winter months in Washington, Oregon, and Idaho. This technique has previously been used to analyze the cytochrome *c* oxidase I gene of *B. cockerelli,* and has identified four psyllid haplotypes. Lso infection was also determined for the psyllids collected from *S. dulcamara*. During both the summer and the winter months in the Pacific Northwest, the Northwestern psyllid haplotype was the predominant population found living on *S. dulcamara*. However, low levels of the Western psyllid population were also present in Washington and Oregon during the same period. No overwintering psyllids tested were Lso-infected, suggesting that these populations do not pose an imminent threat of Lso transmission to newly emerging potatoes and other solanaceous crops in the region, unless a source of Lso becomes available.

**Resumen** *'Candidatus Liberibacter solanacearum'* (Lso) es una bacteria que infecta a cultivos de solanáceas y causa abatimiento y pérdida de cosechas, especialmente en papa y tomate. Lso se transmite a estos hospedantes por el vector psílido de la papa (*Bactericera cockerelli* Sulc). Las plantas hospederas de *B. cockerelli* no se limitan a especies cultivadas, sino que también incluyen muchas malezas silvestres solanáceas. Estas hospedantes silvestres pudieran impactar potencialmente la invernación y apareamiento de los psílidos y servir como reservorios para Lso. En el Noroeste del Pacífico de los Estados Unidos de América se ha reportado recientemente a *B. cockerelli* invernando en la planta "uva del diablo", "dulcamara" o "matagallinas" (*Solanum dulcamara* L.). En el presente estudio se utilizó un análisis de fusión de alta resolución del gen mitocondrial del citocromo c oxidasa de *B. cockerelli* para analizar las poblaciones del psílido que se presentan en *S. dulcamara* durante los meses del verano e invierno en Washington, Oregon y Idaho. Se ha utilizado previamente esta técnica para analizar el gen mencionado, y ha identificado cuatro haplotipos del psílido. También se determinó la infección por Lso en psílidos colectados de *S. dulcamara*. Durante los meses de verano e invierno en el Pacífico del Noroeste, el haplotipo del psílido del Noroeste era la población dominante que se encontraba viviendo en *S. dulcamara*. No obstante, también se encontraba, aunque en bajos niveles de la población, el psílido del Oeste en Washington y Oregon durante el mismo período. Psílidos no invernantes probados estaban infectados de Lso, sugiriendo que estas poblaciones no representan una amenaza inminente de transmisión de

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Lso a papas de nueva emergencia y a otros cultivos de solanáceas en la región, a menos que una fuente de Lso estuviera disponible.

**Keywords** Potato diseases · Liberibacter · Psyllid haplotypes · Nightshade

## Introduction

The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) is a well-known pest of solanaceous crops such as potato (*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.), and pepper (*S. annuum* L.) in North and Central America and New Zealand (Pletsch 1947; Wallis 1955; Al-Jabar 1999; Secor and Rivera-Varas 2004; Munyaneza et al. 2007; Hansen et al. 2008; Liefting et al. 2008a, 2009a; Yang and Liu 2009; Munyaneza 2010, 2012). *B. cockerelli* is a vector of "*Candidatus* Liberibacter solanacearum" (Lso) (also known as "*Candidatus* Liberibacter psyllaurous"), a phloem-limited bacterium linked to plant decline and yield loss in these economically-important solanaceous crops (Hansen et al. 2008; Liefting et al. 2008a, 2009a; Secor et al. 2009). Lso has also been identified in solanaceous weeds such as the cape gooseberry (*Physalis peruviana* L.) in New Zealand, and the silverleaf nightshade (*S. elaeagnifolium* Cav.), wolfberry (*Lycium barbarum* L.), black nightshade (*S. ptychanthum* Dun.), and buffalo bur (*S. rostratum* Dun.) in the central United States (Liefting et al. 2008b, 2009b; Wen et al. 2009; Wen et al. 2010). In addition to being host plants of the potato psyllid, *S. ptychanthum* and *S. rostratum* have been reported as breeding sites for the psyllids (Wallis 1955). Recently, *B. cockerelli* was found breeding and reproducing on bittersweet nightshade (*S. dulcamara* L.) in the Pacific Northwest region of the United States (Murphy et al. 2013).

*B. cockerelli* and its Lso pathogen occurring on wild solanaceous plants are considered a source of concern for growers. Wild solanaceous weeds could act as an overwintering host or breeding site for the psyllids whenever cultivated solanaceous crops are not present (Wallis 1955). In the United States Pacific Northwest, following the initial reporting of the Lso-induced zebra chip (ZC) disease of potato in 2011 (Crosslin et al. 2012a, b), *B. cockerelli* was found overwintering on *S. dulcamara* (Murphy et al. 2013). Specifically, the potato psyllid was found on *S. dulcamara* plants in Washington and Oregon during March and April 2012, and in southwestern Idaho between November 2011 and May 2012. This finding suggests that the wild perennial, *S. dulcamara* can in fact act as an overwintering host plant for the potato psyllid during the colder winter months of the Pacific Northwest.

To date, genetic analyses of *B. cockerelli* have identified four different psyllid populations designated as the Western, Central, Northwestern, and Southwestern haplotypes (Liu et al. 2006; Swisher et al. 2012, 2013a, b, c). Psyllids of the Western haplotype have been found in the western United States, Baja Mexico and New Zealand, while psyllids of the Central haplotype have been found in the central United States, Mexico, and Central America (Liu et al. 2006; Thomas et al. 2011; Swisher et al. 2012, 2013a, c). Psyllids of the Northwestern haplotype have so far only been found in the Pacific Northwestern United States, and psyllids of the Southwestern haplotype have only been reported in northern New Mexico and southern Colorado (Swisher et al. 2012, 2013a, b).

Of particular interest, the Northwestern and Western haplotypes have both been identified within the potato growing regions of Washington, Oregon, and Idaho (Swisher et al. 2012, 2013a). This important region produces more than 55 % of the potatoes in the United States (National Agricultural Statistics Services 2012). The Northwestern haplotype was identified in Washington State as early as 1998, and has persisted in this region since that time (Swisher et al. 2012, 2013a). It was also documented in southwestern Idaho in 2011 (Swisher et al. 2012). The Western haplotype was identified in Washington and Oregon as early as 2008 and has persisted in this region since then (Swisher et al. 2012, 2013a). In 2011, the Western haplotype was also identified in southwestern Idaho (Swisher et al. 2012).

When Lso-induced ZC disease was first reported in the Pacific Northwest during the 2011 potato growing season, psyllids collected from diseased fields were identified as the Western haplotype and had an Lso-infection rate of 5 to 10 % (Crosslin et al. 2012a, b; Swisher et al. 2012; J.M. Crosslin and J.E. Munyaneza unpublished data). Psyllids collected from nearby uninfected fields were predominantly identified as the Northwestern haplotype and were Lso-free. This evidence suggested that it was the Western psyllid population that acquired Lso prior to entering the potato fields, potentially from a long-distance migration from the southwestern United States. Additionally, the limited geographical range of the Northwestern haplotype suggests that the Northwestern psyllids are a local population, perhaps even native to the region.

With the recent finding of *B. cockerelli* overwintering on *S. dulcamara* in the Pacific Northwest, the purpose of this study was to assess the psyllid population(s) occurring on *S. dulcamara* in Washington, Oregon, and Idaho states during the potato growing season, and more importantly, during the winter months. Genetic analysis of the potato psyllids collected from *S. dulcamara* was done using high resolution melting (HRM) analyses of the mitochondrial cytochrome *c* oxidase I gene (CO1). This technique has been used previously to identify the four known potato psyllid haplotypes, and has been supported by DNA sequence analysis (Swisher et al. 2012, 2013a, b, c). In this study, individual psyllids

collected from *S. dulcamara* were also analyzed for the presence of Lso to determine if the overwintering psyllids pose a threat to potato crops in the Pacific Northwest.

## Materials and Methods

*Origin of Psyllids* Overwintering psyllids were collected from two locations in the Yakima Valley of Washington (Zillah and Grandview), and three locations in the southern portion of the lower Columbia Basin near the Oregon and Washington border (Irrigon Wildlife Refuge, Stanfield, and Cold Springs Reservoir) (Table 1). Sampling sites were located in undisturbed areas (state parks and wildlife refuge areas), some of which were selected based on their proximity to potato fields. Psyllids were collected from *S. dulcamara* plants in summer and fall (2012) and during the winter months (2012 to 2013).

Adult psyllids were collected on a weekly basis with a D-VAC device or using the beat-tray technique as described below. In the case of D-VAC sampling, the device was made of a slightly modified Ryobi 2 Cycle Gas Blower-Vac (Ryobi, Model #RY09055, 200 Mph/400cfm). The device was set to the vacuum setting and an organdy bag was inserted at the end of the vacuum tube for insect collection. Insects were collected by walking and holding the D-VAC within 6 in. of the nightshade plants for 2 to 3 min (3 to 4 plants per site). In the case of the beat-tray collection, nightshade foliage was beaten on white board and insects were collected using an aspirator. All samples were placed into a 1 gal plastic bag (Ziploc, Johnston & Son Inc., Racine, WI), taken to the laboratory, and examined for the presence of psyllids using a stereomicroscope. Subsequently, adults were placed into a 1.7 ml graduated microcentrifuge tube (ISC BioExpress, Kaysville, Utah) and stored at −20 °C until further processing. Eggs and nymphs were collected by picking and inspecting nightshade plant foliage.

*Nucleic Acid Extraction* All individual psyllids collected from *S. dulcamara* were subjected to the cetyl trimethyl ammonium bromide (CTAB) nucleic acid extraction method as described in Crosslin et al. (2006).

**Table 1** Coordinates for *S. dulcamara* sampling sites

| Location | Latitude (N) | Longitude (W) |
|---|---|---|
| Zillah, WA | 46° 25.0684′ | 120° 18.3237′ |
| Grandview, WA | 46° 17.3676′ | 119° 57.0801′ |
| Irrigon Wildlife Refuge, OR | 45° 54.665′ | 119° 25.076′ |
| Stanfield, OR | 45° 46.977′ | 119° 13.275′ |
| Cold Springs Reservoir, OR | 45° 52.061′ | 119° 03.068′ |

*Primers, Polymerase Chain Reaction, and DNA Sequencing* A 326-bp portion of the *B. cockerelli* mitochondrial CO1 gene was utilized for the HRM analysis of all individual psyllids using the primers, CO1 meltF and CO1 353R (Chapman et al. 2012; Swisher et al. 2013a). This amplicon was previously used for HRM analysis by Swisher et al. (2013a). For DNA sequencing analysis, a 500-bp portion of CO1 that encompasses the 326-bp CO1 amplicon was generated using the *B. cockerelli*-specific primers, CO1 F3 and CO1 R3 (Crosslin et al. 2011). This amplicon has been used previously for DNA sequencing in Swisher et al. (2012, 2013a, b, c). For all DNA sequencing analysis, conventional polymerase chain reaction (PCR) was used as described in Crosslin et al. (2011). PCR products were then purified using the Wizard PCR Clean Up Kit (Promega, Madison, Wisconsin) and subjected to direct DNA sequencing using the CO1 F3 and CO1 R3 primers.

Following DNA extraction of individual psyllids, samples were subjected to conventional PCR analysis for the detection of Lso. Negative controls consisting of a water-only check and Lso-negative psyllid DNA, in addition to positive controls comprised of Lso-positive psyllid DNA were used in all PCR analyses. Primers OA2 and OI2c were used to detect the Lso bacterium as described previously in Crosslin et al. (2011). PCR products were visualized on a 1.5 % agarose gel stained with ethidium bromide. Presence of the predicted 1168-bp amplicon indicated a sample was positive for Lso. For all analyses where sample number was greater than 10 psyllids per collection date per location, sample number was limited to 10 psyllids. These samples totaled 210 psyllids collected between May and October 2012, and 309 psyllids collected in April 2012 and between November 2012 and April 2013.

*High Resolution Melting Analysis* HRM analysis of the 326-bp CO1 amplicon of all individual potato psyllids was done using the LightCycler 480 and the corresponding LightCycler 480 Gene Scanning software (Roche Applied Science, Indianapolis, Indiana) as described previously in Swisher et al. (2013a).

## Results

### High Resolution Melting Analysis

Individual potato psyllids collected from *S. dulcamara* were analyzed by HRM of a partial mitochondrial CO1 gene to identify the specific psyllid populations living on the host plant in Washington, Oregon, and Idaho. Specifically, the melting profile of a 326-bp CO1 amplicon was used to differentiate the Western and Northwestern haplotypes from the Central and Southwestern haplotypes. This amplicon generates a single-peak melting profile for the Northwestern, Central, and

Southwestern haplotypes, whereas the Northwestern peak is distinguished by a shift in the melting peak to a lower temperature. The Central and Southwestern melting peaks overlap and are therefore mostly indistinguishable from each other. The Western haplotype shows a double-peak melting profile shifted to a higher temperature (Swisher et al. 2013a). Samples from previously published analyses were used as controls for each haplotype (Swisher et al. 2012, 2013b; Fig. 1, dashed lines). The raw fluorescent data was analyzed with the LightCycler 480 Gene Scanning software (Roche Applied Science) to generate the normalized melting curves and the melting peaks (Fig. 1a and b). HRM analysis of a representative sampling of psyllids collected from *S. dulcamara* near Stanfield, Oregon, identified psyllids from both the Northwestern and Western haplotypes (Fig. 1, solid lines).

In total, 210 individual psyllids collected from *S. dulcamara* in Washington and Oregon during the potato growing season (May to October) were analyzed by HRM using the 326-bp CO1 amplicon (Fig. 2 and Table 2). From collections made near Zillah, Washington, between June 11, 2012 and October 29, 2012, 125 psyllids were analyzed. Of these psyllids, 123 were identified as the Northwestern haplotype. The remaining 2 psyllids, collected mid-September, were identified as the Western haplotype. In nearby Grandview, Washington, 48 psyllids were analyzed by HRM from collections made between July 23, 2012 and October 29, 2012. Of these psyllids, 47 were identified as the Northwestern haplotype, while 1 psyllid collected in late October was identified as the Western haplotype. At three sites near Hermiston, Oregon, (Irrigon Wildlife Refuge,

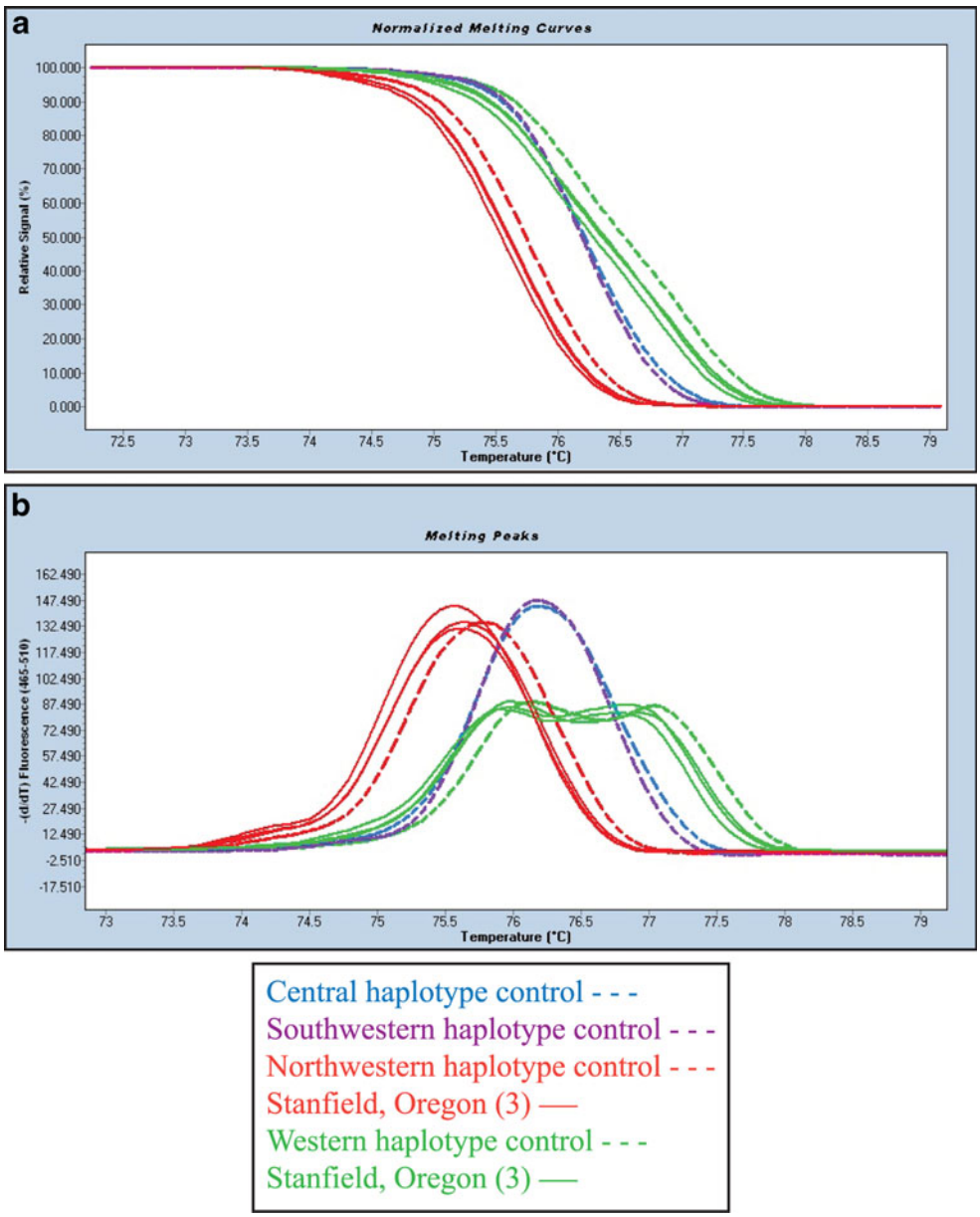


**Fig. 1** HRM analysis of a 326-bp partial CO1 amplicon. The normalization of the raw fluorescent data (**a**) and the melting peaks generated (**b**) distinguish the Northwestern and Western haplotypes from the Central and Southwestern haplotypes. Previously published samples of the Northwestern, Central, Southwestern, and Western haplotypes were used as controls and identified by the dashed lines in Red, Blue, Purple, and Green, respectively (Swisher et al. 2012, 2013b). HRM analysis of a representative sampling of overwintering psyllids collected near Stanfield, Oregon, identified a mix of Northwestern (3) and Western (3) haplotype psyllids (solid lines in red and green, respectively)

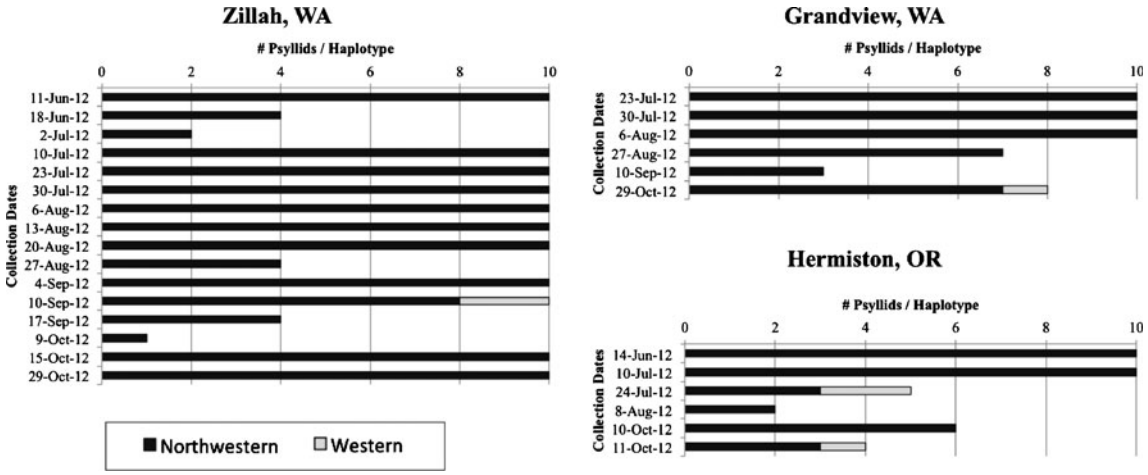


**Fig. 2** Summary of HRM results from psyllids collected from *S. dulcamara* during the potato growing season (May to October) in Washington and Oregon. In total, 210 psyllids collected over the course of the potato growing season from *S. dulcamara* located near Zillah and Grandview, Washington, and Hermiston, Oregon, were analyzed by HRM of the 326-bp CO1 amplicon. These psyllids were identified as the Northwestern and Western haplotypes

Stanfield, and the Cold Springs Reservoir), 37 psyllids were analyzed by HRM from collections made between June 14, 2012 and October 11, 2012. Of these psyllids, 34 were identified as the Northwestern haplotype and 3 were identified as the Western haplotype. The 3 psyllids from the Western population were collected in late July near Stanfield, Oregon (2), and mid-October near the Cold Springs Reservoir in Oregon (1).

Overwintering psyllids were collected in Washington, Oregon, and Idaho from *S. dulcamara* in April 2012 and between the months of November 2012 and April 2013, totaling 309 individual psyllids (Fig. 3 and Table 2). In Zillah, Washington, a total of 73 overwintering psyllids collected between November 2012 and January 2013 were analyzed by HRM of the 326-bp CO1 amplicon. All of these psyllids were identified as the Northwestern haplotype. From collections made near Grandview, Washington, 51 psyllids were analyzed. Of these psyllids, 50 were identified as the Northwestern haplotype, while 1 psyllid collected on January 15, 2013 was identified as the Western haplotype. In northeastern Oregon, 168 overwintering psyllids were analyzed by HRM from collections made at three different sites near Hermiston. West of Hermiston, located along the Columbia River near the Irrigon Wildlife Refuge, 86 overwintering psyllids were collected from *S. dulcamara* in April 2012 and between November 2012 and April 2013. All of these psyllids were identified as the Northwestern haplotype. South of Hermiston, near Stanfield, Oregon, 51 psyllids were analyzed from collections made between November 2012 and February 2013. Of these psyllids, 45 were identified as the Northwestern haplotype, while 6 were identified as the Western haplotype. These 6 psyllids of the Western haplotype were collected in the months of November, December, and January. East of Hermiston, near the Cold Springs Reservoir, 31 psyllids were analyzed from collections made between November 2012 and April 2013. Of these psyllids, 30 were identified as the Northwestern haplotype and 1 psyllid, collected in mid-November, was identified as the Western haplotype. In southwestern Idaho, 17 overwintering psyllids were analyzed from collections made near Star and Eagle in April 2012, December 2012, and February 2013. All of these psyllids were identified as the Northwestern haplotype.

**Table 2** Haplotyping results of *B. cockerelli* collected from *S. dulcamara*

| | Location | # Northwestern Haplotype | # Western Haplotype |
|---|---|---|---|
| Summer | Zillah, WA | 123 | 2 |
| | Grandview, WA | 47 | 1 |
| | Hermiston, OR | 34 | 3 |
| Winter | Zillah, WA | 73 | 0 |
| | Grandview, WA | 50 | 1 |
| | Irrigon Wildlife Refuge, OR | 86 | 0 |
| | Stanfield, OR | 45 | 6 |
| | Cold Springs Reservoir, OR | 30 | 1 |
| | Star and Eagle, ID | 17 | 0 |

## DNA Sequencing Analysis

To verify the HRM results obtained from analysis of individual potato psyllids collected from *S. dulcamara*, DNA sequencing analysis was performed on 20 individual psyllid samples collected from the different locations in Washington and Oregon. A 500-bp CO1 amplicon that includes the 326-bp CO1 amplicon used in the HRM analyses was utilized for sequencing. All sequencing results were compared to the

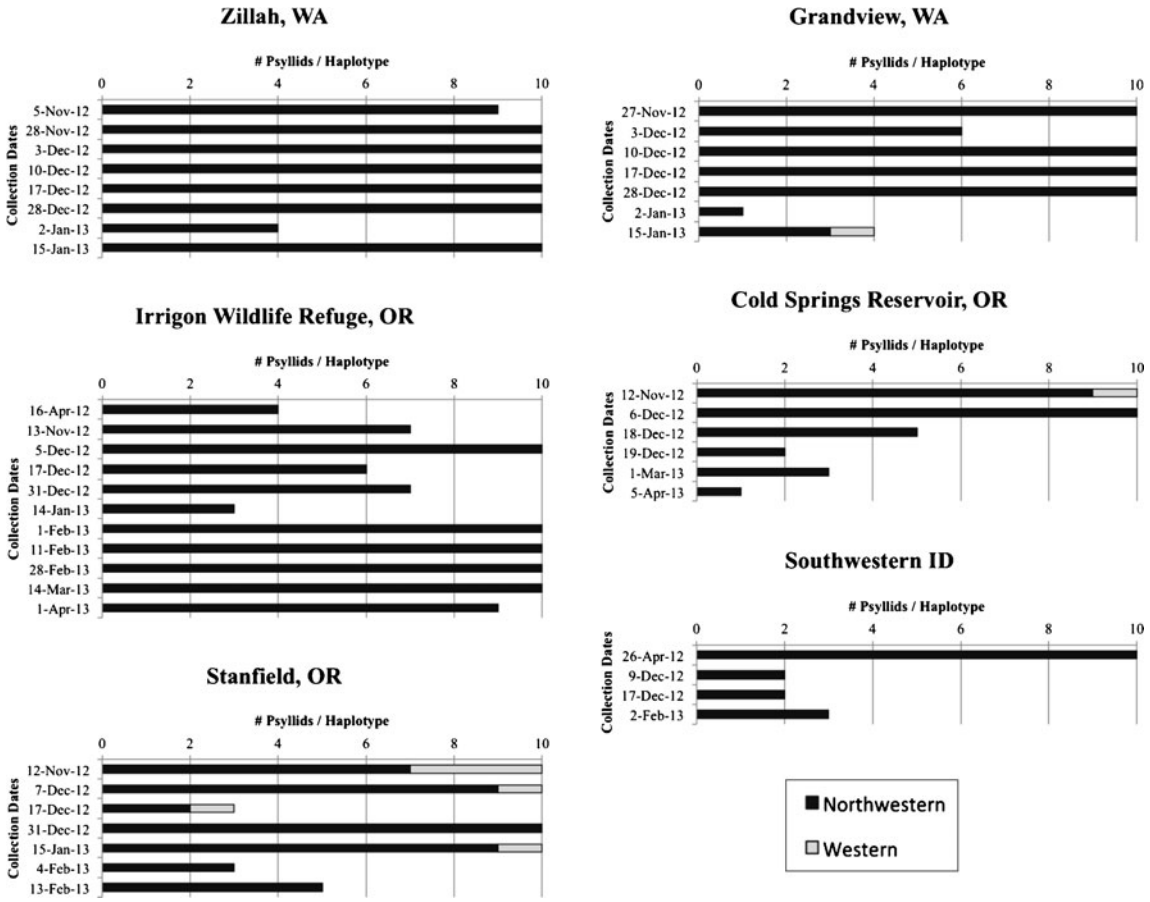


**Fig. 3** Summary of HRM results from psyllids collected from *S. dulcamara* during the winter months (November to April) in Washington, Oregon, and Idaho. In total, 309 psyllids collected during the winter months from *S. dulcamara* located near Zillah and Grandview, Washington, Eagle and Star, Idaho, and the Irrigon Wildlife Refuge, Stanfield, and the Cold Springs Reservoir, Oregon, were analyzed by HRM of the 326-bp CO1 amplicon. These psyllids were identified as the Northwestern and Western haplotypes

known sequences of the Northwestern and Western haplotypes (data not shown, GenBank accession numbers JQ708093 and JQ708095, respectively; Swisher et al. 2012). Seventeen psyllids designated as the Northwestern haplotype by HRM analysis were confirmed as Northwestern by DNA sequencing (9 overwintering psyllids and 8 psyllids collected during the potato growing season). The remaining 3 psyllids analyzed by DNA sequencing were designated as the Western haplotype by HRM analysis (1 overwintering psyllid and 2 psyllids collected during the potato growing season). These samples were confirmed as the Western haplotype by DNA sequencing results.

### Analysis of Lso Infection

To test if psyllids living on *S. dulcamara* during the potato growing season are a source of Lso for neighboring solanaceous crops, conventional PCR was used to analyze the 210 *B. cockerelli* samples originating from collections made between May and October 2012. A single psyllid of the Western haplotype, collected on the 24$^{th}$ of July, 2012 from a *S. dulcamara* plant located near Stanfield, Oregon, tested positive for Lso infection. No other psyllids analyzed by HRM from collections made throughout the summer were positive for Lso.

Similarly, the presence of overwintering potato psyllids on *S. dulcamara* in the Pacific Northwest raises significant concerns about whether these psyllids provide an overwintering host for the Lso bacterium. To assess the potential risk these overwintering psyllids might have on cultivated crops emerging in the late spring, conventional PCR was used to analyze the 309 individual *B. cockerelli* samples originating from collections made in April 2012 and between November 2012 and April 2013 for Lso infection. No overwintering psyllids tested positive for Lso.

## Discussion

Until recently, it was generally believed that the potato psyllid did not overwinter in the Pacific Northwest (Munyaneza et al. 2009). However, in late 2011 and early 2012, the potato

psyllid was found living on the perennial weed, *S. dulcamara* in this region (Murphy et al. 2013). In the present study, HRM analysis of a partial CO1 amplicon identified two haplotypes of the potato psyllid living on *S. dulcamara* during both the summer (potato growing season) and the winter seasons in Washington, Oregon, and Idaho. Psyllids of the Northwestern haplotype predominated in this region throughout both seasons, but psyllids of the Western haplotype were also present in low numbers. No psyllids of the Central or Southwestern haplotypes were identified from psyllids collected from *S. dulcamara* in this region.

HRM analyses identified 97.4 % of the *S. dulcamara* overwintering population in Washington, Oregon, and Idaho as the Northwestern haplotype. Psyllids of the Northwestern haplotype can therefore survive the cold Pacific Northwestern winter temperatures in this region, with lows that reach well below freezing temperatures for much of December, January, and February. Interestingly, since the Northwestern haplotype has yet to be identified outside of the Pacific Northwest, it is possible that the cold-tolerance of these psyllids allow them to maintain a local population year-round. Additional biological studies are needed to compare cold-hardiness of all four psyllid haplotypes to determine if the Northwestern haplotype has a higher level of cold-tolerance than the other psyllid haplotypes.

Of particular interest, results from this study suggest that psyllids of the Northwestern haplotype find *S. dulcamara* plants to be an adequate host during both the winter and summer months. These results also suggest that psyllids of the Western haplotype may not prefer the *S. dulcamara* plant as a host. For example, of 205 psyllids tested from collections made on *S. dulcamara* during the summer and winter months near Hermiston, Oregon, only 4.9 % were identified as the Western haplotype. In this same region, 121 psyllids collected in or next to potato fields at the end of August and beginning of September 2012 were analyzed by HRM, 71.1 % of which were identified as the Western haplotype and 28.9 % as the Northwestern haplotype (data not shown). These results suggest that the Western haplotype may show a preference for solanaceous crops over *S. dulcamara* in northeastern Oregon. Further studies are required to validate this hypothesis.

The identification of *S. dulcamara* as a suitable host for potato psyllids to survive the cold winter months in the Pacific Northwest raises the question of whether Lso-positive psyllids can survive on *S. dulcamara*. Interestingly, only one Lso-infected psyllid was found from all 519 psyllids tested, and this psyllid was collected from *S. dulcamara* during the potato growing season, not during the winter season. This result suggests that Lso-infected potato psyllids may not have survived the winter months on *S. dulcamara* during the 2011 to 2012 and 2012 to 2013 winters in the Pacific Northwest. Furthermore, this suggests that the *S. dulcamara* overwintering psyllids should not be considered a reservoir of Lso or a threat to newly emerging crops during the springtime, provided an additional Lso source does not become available.

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