EXPLORING THE ASSOCIATION OF POTATO PSYLLIDS AND *CANDIDATUS* LIBERIBACTER SOLANACEARUM WITH NATIVE SOLANACEAE IN A CALIFORNIA DESERT ECOSYSTEM

By

Natalie Anne Chan

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University of California, Riverside

APPROVED

Dr. Kerry Mauck

Department of Entomology

Dr. Richard Cardullo, Howard H Hays Jr. Chair

University Honors

ABSTRACT

Understanding the interactions between vector-borne pathogens and non-crop plant species is crucial for managing diseases in both agricultural and natural ecosystems. This study focuses on the role of potato psyllid, *Bactericera cockerelli*, haplotypes, and the pathogen *Candidatus* Liberibacter solanacearum, CLso, in affecting native plants in the genera *Physalis* and *Lycium* within California's Mojave and Sonoran Deserts. We employed a combination of yellow sticky cards and custom-built preservative traps to monitor psyllid populations and the bacterial pathogens they may carry over the late winter to early spring season. Molecular techniques, including PCR and Sanger sequencing, were used to identify psyllid haplotypes and screen each psyllid for infection with CLso. Our results indicated that the Southwestern haplotype of potato psyllids was prevalent, but none of the captured psyllids tested positive for CLso. These findings suggest a currently low risk of CLso transmission to native desert plants by this vector in the studied regions. However, the presence of another psyllid species, *Bactericera dorsalis*, which has been identified as a potential vector for CLso, underscores the complexity of these ecological interactions and highlights the need for ongoing surveillance. This study provides valuable insights into the dynamics of vector-borne pathogens in non-crop plants, contributing to a broader understanding of their ecological impacts and aiding in the development of integrated pest management strategies.

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INTRODUCTION

Due to its relevance for food security, there is extensive research on vector-borne pathogens and their impacts on crop plants. This is the case for a pathosystem consisting of the potato psyllid vector (*Bactericera cockerelli*), and *Candidatus* Liberibacter solanacearum (CLso), the bacterial pathogen it transmits to potatoes, tomatoes, and other solanaceous crops *Candidatus* Liberibacter solanacearum (CLso), has been extensive (Munyaneza, 2015). However, there remains a significant gap in our understanding of how CLso and other vector borne pathogens affect non-crop host plants, and even whether they are moving into non-crop habitats (Malmstrom et al., 2016). This oversight is particularly pertinent considering the potential implications for conservation and restoration efforts. Knowing whether pathogens and vectors are spilling over from crop habitats will provide insights into ecosystem resilience and the cascading effects of disease outbreaks on plant communities and associated fauna. Understanding the interactions between vector-borne pathogens and non-crop plants in preserved natural areas is crucial for mitigating the impacts of human activities, such as agriculture, on natural communities. The work presented here addresses this knowledge gap for the CLso-potato psyllid pathosystem by studying psyllid-host interactions and CLso infection status in a large preserved natural habitat: Joshua Tree National Park.

Josh Tree National Park

Joshua Tree National Park (JTNP) is home to a diverse range of unique ecosystems and endemic species, including plant families such as *Solanaceae*, which are known host plants for potato psyllids. This makes it an ideal location to study the interactions between this vector and native plant populations, providing insights into how the associated pathogen, *Candidatus*

Liberibacter solanacearum, might impact non-crop species and potentially disrupt local biodiversity.

Biology of the potato psyllid: *Bactericera cockerelli*

Bactericera cockerelli is a member of the family Triozidae within the superfamily Psylloidea (psyllids) and order Hemiptera. Within this species, there is considerable genetic diversity, as evidenced by the presence of multiple genetic haplotypes: Northwestern, Western, Central, and Southwestern. As seen in Table 1, these haplotypes show varying distributions across different geographical regions, reflecting the genetic structure and evolutionary history of potato psyllid populations.

Haplotype	Distribution	Host plants	References
Southwestern	California, Colorado, Idaho, New Mexico, Texas	Potatoes	Dahan et al., 2017, Jaimie et al., 2024, Swisher et al., 2014a, Swisher et al., 2014b, Workneh et al., 2018.
Central	Colorado, El Salvador, Honduras, Idaho, Kansas, Mexico, Nebraska, New Mexico, Nicaragua, North Dakota, SouthwestUS, Texas, Washington, Wyoming	Peppers, potatoes, tomatoes	Dahan et al., 2017, Liu & Trumble, 2007, Liu, Trumble & Stouthamer, 2006, Swisher et al., 2013, Swisher et al., 2014a, Swisher et al., 2013, Workneh et al., 2018
Western	Baja, California, Colorado, Idaho, Kansas, Mexico, Nebraska, New Mexico, Oregon, Texas, Washington	*Lycium barbarum*, pepper, potatoes, potatoes, *Solanum dulcamara*, tomato	Cooper et al., 2022, Dahan et al., 2017, Liu & Trumble, 2007, Liu, Trumble & Stouthamer, 2006, Swisher & Crosslin, 2014, Swisher et al., 2013, Swisher et al., 2014a, Swisher et al., 2014b, Swisher, Munyaneza & Crosslin, 2012, Swisher, Munyaneza & Crosslin, 2013, Thinakaran et al., 2017, Workneh et al., 2018.

Northwestern	California, Idaho, Oregon, Washington	*Lycium barbarum*, potatoes, *Solanum dulcamara*, tomatoes	Dahan et al., 2017, Jaimie et al., 2024, Swisher et al., 2013, Swisher et al., 2014a, Swisher et al., 2014b, Swisher, Munyaneza & Crosslin, 2012, Swisher, Munyaneza & Crosslin, 2012, Swisher, Munyaneza & Crosslin, 2013, Thinakaran et al., 2017.

Table 1. The four haplotypes of the potato psyllid including their distribution, host plants, and references consulted.

Potato psyllids feed by piercing into the plant phloem tissue with their stylets, which are specialized mouthparts adapted for navigating between plant cells to reach plant vasculature. After reaching the phloem, the insect ingests sugary sap that flows within the phloem sieve tube elements. As shown in Table 1, potato psyllids are specialized in specific genera of plants in the family Solanaceae. This specialization in host plant selection is mediated by chemical cues emitted by the plants, which attract the psyllids and guide them to suitable feeding sites. Once they have located a suitable host plant, potato psyllids may remain stationary for extended periods, continuously feeding on the phloem sap. This feeding activity not only provides the psyllids with essential nutrients but also facilitates the transmission of bacterial plant pathogens such as CLso, which colonize the phloem sieve tube elements after entering the plant via psyllid salivation (Muack et al., 2024, Wenninger et al., 2023).

The historical perspective of potato psyllid outbreaks in the western USA reveals that these potato psyllid outbreaks are not only recent concerns but have deep roots in the region's agricultural history. As Mauck et al. (2019) highlighted, a previously unidentified haplotype of Ca. Liberibacter psyllaurous was found in a 49-year-old herbarium specimen of *Solanum umbelliferum*, a native host plant. This discovery underscores the long-standing presence of the

pathogen in native ecosystems before it emerged as a significant agricultural pest with the first reported cases of the zebra chip disease in potato crops in the early 2000s.

Components like environmental factors, host plant availability, and intrinsic biological characteristics of the psyllid contribute to its pest status (Wenninger et al., 2023). The psyllid's life cycle is closely tied to solanaceous plants, where it completes multiple generations per year, facilitated by mild winters and abundant host plants in agricultural and native habitats. This adaptability has enabled potato psyllids to maintain continuous populations that move between crops and non-crop hosts, complicating management efforts.

Moreover, the management strategies for controlling potato psyllids and mitigating zebra chip disease, as discussed by Wenninger et al., (2023) include both traditional approaches such as insecticides and cultural practices, alongside more innovative methods such as host plant resistance and biological control. The complexity of managing this pest is exacerbated by its ability to rapidly develop resistance to chemical pesticides and its wide range of host plants spanning across different ecosystems.

Biology of *Candidatus* Liberibacter solanacearum (CLso)

Candidatus Liberibacter solanacearum is a fastidious, phloem-limited, gram-negative bacterium belonging to the Liberibacter genus within the family Rhizobiaceae. This pathogen infects and causes disease in several economically important crops in the family Solanaceae, including potatoes, tomatoes, and peppers. One of the diseases caused by CLso in potatoes is the zebra chip disease, a serious issue for the potato industry due to its impact on both yield and quality. This disease reduces potato yields by 50-85% in all regions where this pathosystem is found (Greenway et al., 2014, Greenway et al., 2018). As depicted in Figure 2, the disease is characterized by streaks or stripes in potato tubers that darken when fried, thus affecting the

commercial value of potatoes. The pathogen's effect on plant health is multifaceted, impacting photosynthesis, nutrient allocation, and overall plant vigor (Prager et al., 2022). The symptomatic expression of the disease, including leaf curling, yellowing, and eventual necrosis, underscores the pathogen's severe impact on crop physiology.

CLso, however, cannot be cultured in the lab and is unable to survive outside its psyllid or plant hosts (Kenney et al., 2024). Its small genome size, high sequence diversity, and dependence on host plants and insect vectors like the potato psyllid complicate efforts to culture it in vitro or manipulate it genetically. The mobility of the potato psyllids enables them to acquire the pathogen while feeding on infected plants and transmit it to healthy plants across various life stages, thus facilitating the rapid spread of CLso across fields and making the management of the disease particularly challenging (Wenninger et al., 2022). Adding to these challenges is the bacterium's primary residency within the phloem tissue of infected plants, which complicates its detection and study. Despite these challenges, understanding the biology and transmission dynamics of CLso is crucial for developing effective management strategies to mitigate its impact on both agricultural and natural ecosystems.

Management strategies are limited, as CLso is unculturable with current laboratory techniques, severely hindering research into its biology and control. Although genetic manipulation of CLso might allow for the development of resistant potato varieties, this remains theoretical due to the pathogen's small genome and high genetic diversity. In terms of control measures, breeding for resistance or tolerance seems promising. Prager et al. (2022) stress the importance of identifying and breeding potato varieties that can resist psyllid infestation or tolerate the pathogen without severe disease manifestation. However, such breeding programs are long-term endeavors that require significant investment and time to yield results.

Meanwhile, integrated pest management strategies are the most immediate approach to mitigating the impact of CLso. According to Wenninger et al. (2023), integrated pest management strategies include careful monitoring of psyllid populations, timely application of insecticides, and the use of cultural practices such as crop rotation and barrier crops. Nonetheless, the adaptability of the potato psyllid and its evolving resistance to common insecticides complicates these efforts.

Moreover, recent findings indicate that CLso can infect non-crop host plants and is present in native ecosystems (Kenney et al., 2024). This further complicates the management scenario. This broad host range suggests that eradication of CLso from agricultural settings might not prevent its reintroduction from nearby wild reservoirs, thus necessitating a landscape-level approach to disease management that considers both agricultural and natural ecosystems.

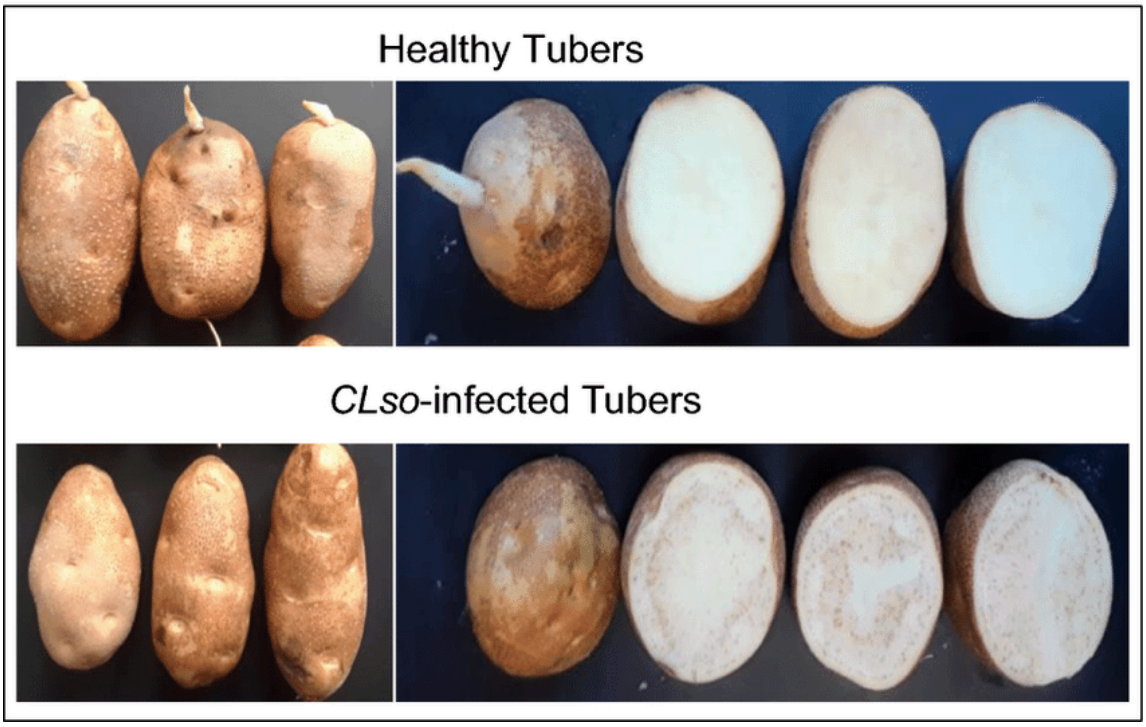

Figure 2. Comparison of tubers in the Solanaceae family from healthy and CLso-infected plants. (Kumar et al., 2022).

Understanding the dynamics of vector-borne pathogens, particularly focusing on the potato psyllid and CLso, is critical for understanding potential impacts on both crop and non-crop plants (Munyaneza et al., 2012). Previous research has identified associations between particular genetic variants (haplotypes) of CLso and particular haplotypes of potato psyllids (Cooper et al., 2015). Notably, a haplotype of CLso was discovered in herbarium specimens of native *Solanum umbelliferum* plants that were collected decades before the first detections of CLso in diseased U.S. potato crops (Mauck et al., 2019). Kenney et al. (2024) subsequently found that this "wild" haplotype, which was named CLso haplotype G, is still found infecting contemporary *S. umbelliferum* and the Northwestern and Southwestern haplotypes of potato psyllids. CLso haplotype G is also different genetically from CLso haplotypes usually causing disease in crops. Furthermore, the study by Kenney et al. (2024) expands this insight by confirming that CLso is not only present outside of cultivated crops in wild hosts like S. umbelliferum but also in other abundant members of plant communities in unique desert habitats. This highlights the need to examine these additional host plants for evidence of CLso haplotypes, which could influence broader ecological and plant health dynamics in the Mojave and Sonoran Deserts. This study system provides a unique opportunity to examine the interactions between vector-borne pathogens and non-crop plant species in California's Mojave and Sonoran Deserts native ecosystems, shedding light on the broader implications for plant health and ecosystem stability.

Understanding CLso and potato psyllid movement among desert Solanaceae

To understand the dynamics of potato psyllids and CLso in the Mojave and Sonoran deserts, we focused on monitoring potato psyllid activity in relation to native plant species in the genera *Physalis* and *Lycium* growing in Joshua Tree National Park. The selection of these species in this particular region was motivated by evidence suggesting associations between potato psyllids and CLso with plants in these genera in other geographic regions dominated by different haplotypes of potato psyllids and CLso. For instance, in the eastern portion of the potato psyllid's range in North America, including Texas and Mexico, Central haplotype potato psyllids have been found on native *Lycium andersonii* and *Lycium cooperi*. (Cooper et al., 2022; Thinakaran et al., 2017). Furthermore, CLso has been detected infecting some of these *Lycium* species (Delgado-Luna et al., 2023; Reyes Corral et al., 2020). In addition, potato psyllids and CLso have also been found to associate with *Physalis* species such as *Physalis crassifolia*, which can be found in Joshua Tree National Park (Delgado-Luna et al., 2023). There is a need to investigate the potential movement of potato psyllids from solanaceous crop areas in the Coachella Valley, adjacent to Joshua Tree National Park, into the park itself. Currently, it's unclear whether this migration is occurring, which specific psyllid haplotypes might be involved, and whether these psyllids are interacting with potential host plants within the park. This study aims to fill these knowledge gaps, assessing the risk to both agricultural areas and native desert ecosystems.

We hypothesized that potato psyllids are moving from agricultural areas in the Coachella Valley into Joshua Tree National Park and interacting with native Solanaceae species. To test this hypothesis, we sampled potential host plants in the park, focusing on identifying whether potato psyllids visit these native desert perennials, specifically in the genera *Lycium* and *Physalis*, during their late winter-early spring growing season.

This study addresses the knowledge gap regarding the association of potato psyllids and CLso with native Solanaceae in the Mojave and Sonoran deserts. We aimed to investigate whether potato psyllids visit and feed on native desert perennials in the genera *Lycium* and *Physalis* during their late winter-early spring growing season, potentially transmitting CLso to these plants. Our hypothesis posited that potato psyllids visit native *Lycium* and *Physalis* species in the Mojave and Sonoran desert and that they may carry CLso, posing a risk of infection to these unique desert plants and leading to possible negative health impacts. To test this hypothesis, we employed two trapping methods to monitor *Lycium andersonii, L. cooperi, Nicotiana obtusifolia,* and *Physalis crassifolia* in Joshua Tree National Park for potato psyllid visitation throughout one late winter to early spring growing season. We then utilized PCR and Sanger sequencing to screen the captured potato psyllids for CLso infection and determine their mitochondrial haplotype. This study is unique in its focus on native desert plant species and their interaction with potato psyllids and CLso in a region previously overlooked in similar investigations.

METHODS

Psyllid sampling

Before going to collect potato psyllids at the Joshua Tree National Park, graduate student mentor Jaimie Kenney obtained the necessary scientific research permits to set up traps and collect insect specimens within the boundaries of the national park. She used iNaturalist observations to select potential trapping sites where one or more of the three target host plant species were present. She then scouted these potential trapping sites on foot to assess the phenology of the target host plants at each location. We selected five final trapping sites (Table 3

and Figure 4) based on whether the target psyllid host plant species were in the leaf. We then identified the plants to place the two types of traps on it; traditional yellow sticky cards and custom-built psyllid preservative traps shown in Figure 5. Traps were set up at each of these five locations, one of each type on each host plant species. After placing the traps, I visited JTNP every two weeks with my graduate student research mentor to change out the psyllid traps and perform active psyllid surveys. Active psyllid surveys included shaking the plants over a canvas beating sheet and capturing any psyllids with an insect aspirator shown in Figure 3. The potato psyllids on the yellow sticky cards and the preservative traps were then stored in Ziploc bags in a fridge at 4℃.

Location	Coordinates of the location	Plants
Cottonwood Springs Road	33.71772385, -115.8086319	*Physalis crassifolia, Lycium andersonii*
Cottonwood Spring	33.73646164, -115.8105545	*Lycium andersonii, Lycium cooperi*
Smoketree Wash	33.801548, -115.7808456	*Lycium andersonii, Lycium cooperi, and Nicotiana obtusifolia*
Porcupine Wash	33.84611893, -115.7782974	*Physalis crassifolia, Lycium andersonii*
Wilson Canyon	33.93652344, -115.9648285	*Lycium andersonii*

Table 3. The locations, the GPS coordinates of each location, and the plants that psyllid sampling was done at each location.

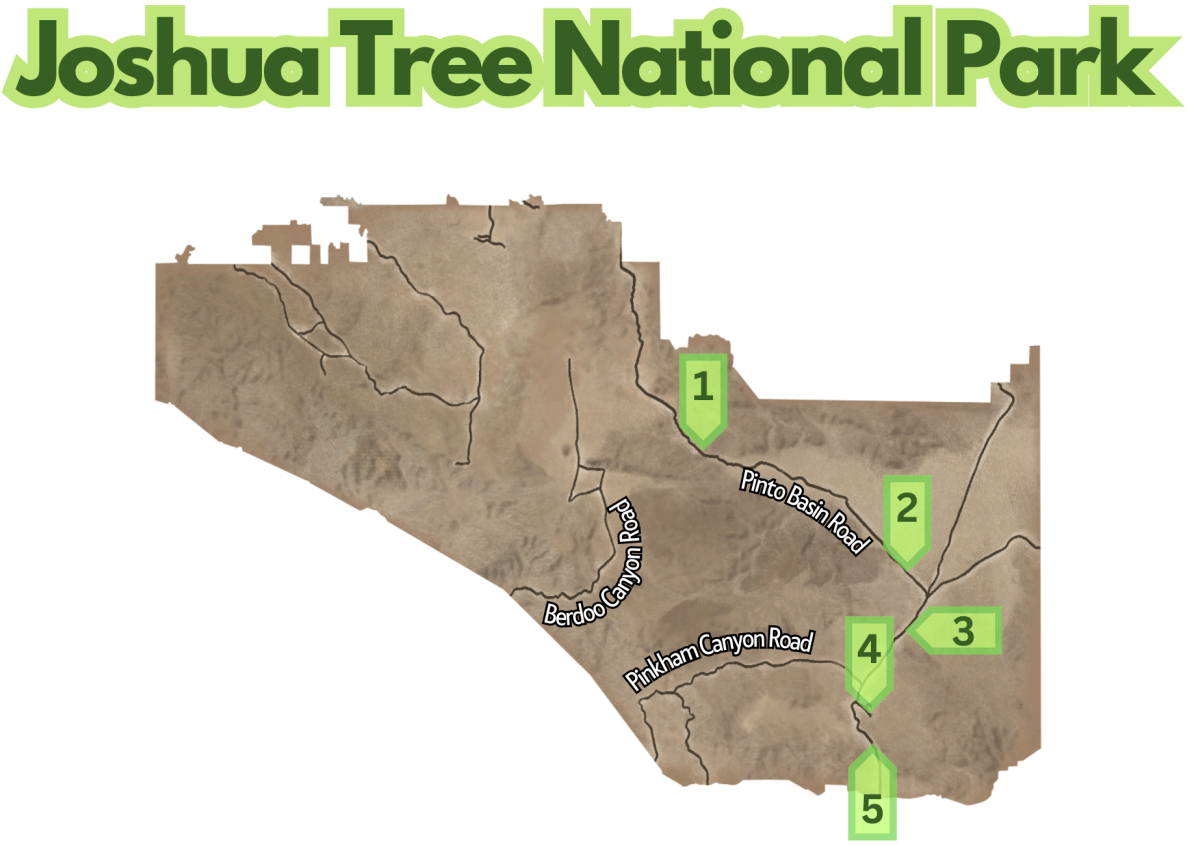

Figure 4. Map of all of the location names and GPS coordinates of psyllid sampling sites in Joshua Tree National Park. The numbers on the map correlate to sampling location; 1 is Wilson Canyon, 2 is Porcupine Wash, 3 is Smoketree Wash, 4 is Cottonwood Spring, and 5 is Cottonwood Spring Road.

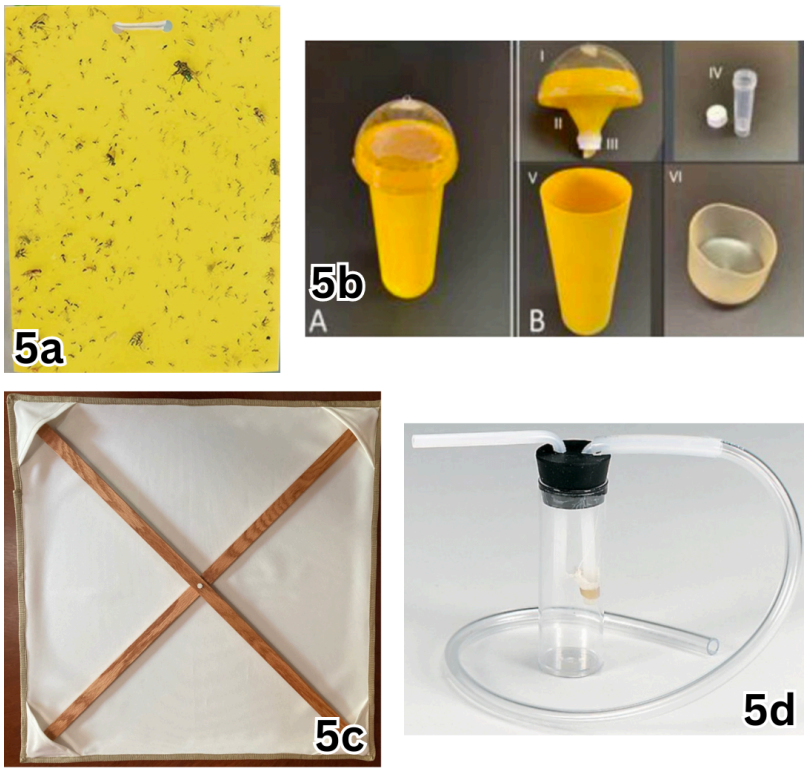

Figure 5. All of the sampling materials used in this study. Inset image 5a shows a traditional yellow sticky card with captured insects. Inset image 5b shows a custom-built psyllid preservative trap fully assembled (A) or disassembled to parts (B) Inset image 5c shows a canvas beating sheet along with the aspirator used to remove insects from the canvas surface (5d).

Potato psyllid identification

Psyllids captured in traps were examined under a dissecting microscope and identified based on key morphological features such as wing venation and dorsal markings, as shown in Figure 6, to ensure accurate species identification. Occasionally, specimens of a related species, *B. dorsalis*, were found on the sticky cards; however, the majority of the captured psyllids were identified as *B. cockerelli*. We quantified the total number of psyllids of each species captured on each card to assess their host plant preferences.

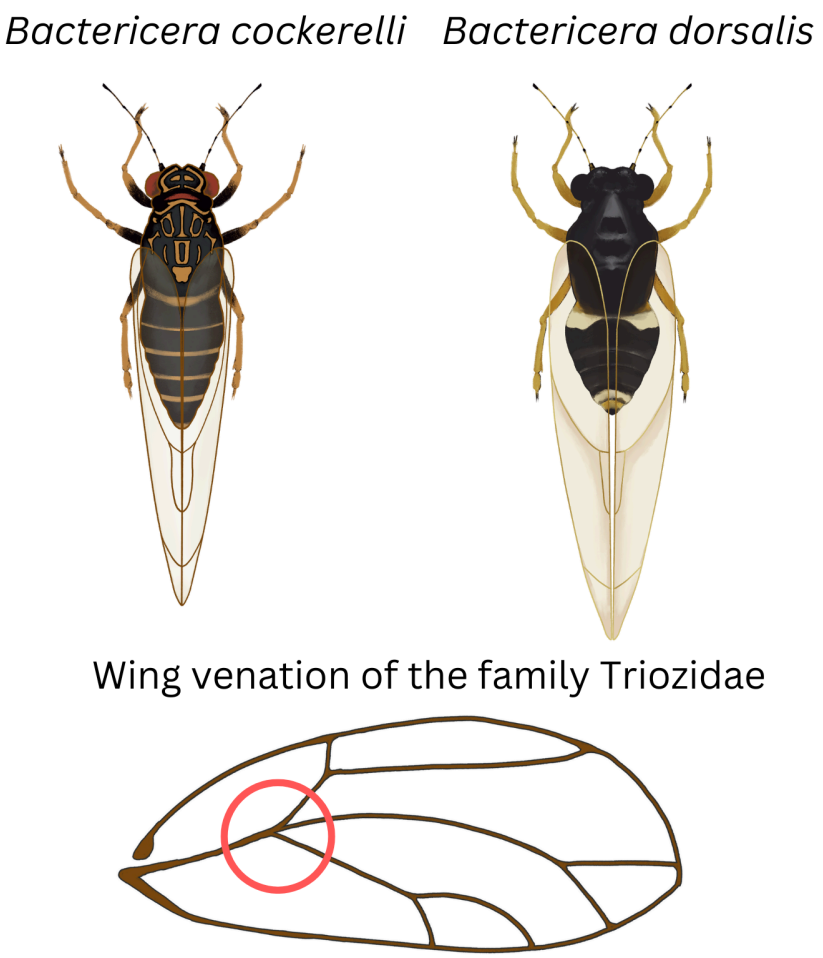

Figure 6. Dorsal view of adult *Bactericera cockerelli* and *Bactericera dorsalis*. Dorsal view of the wing venation of the family Triozidae which includes *B. cockerelli* and *B. dorsalis*. *B. dorsalis* is larger than *B. cockerelli* and has wings approximately twice its body length. *B. cockerelli* also has a light brown symmetrical pattern on its head and thorax that can be seen on the dorsal view. The red circle includes the wing venation identified insects in the family Triozidae.

DNA extraction

Up to 10 potato psyllids were carefully removed from each yellow sticky card for DNA extraction, with one card per host plant, location, and time point). Prior to extraction, grinding pestles and workbench spaces were sterilized with 10% bleach to ensure the DNA from the samples was not contaminated. The psyllids were placed in individual 1.7 ml tubes and then immersed in liquid nitrogen to make the psyllid body easier to grind using the pestles until they

turned into fine powder. DNA was isolated from individual psyllids using the Qiagen DNeasy Blood & Tissue Kit. The kit was used according to the manufacturer's recommendations for insect specimens, with one modification: the volume of the AE elution buffer was decreased to 50 uL to ensure a higher concentration of DNA in the final eluate. A Thermo Scientific NanoDrop™ 2000 Spectrophotometer was used to measure the total DNA concentration and purity.

Amplification of barcoding genes using PCR

PCR (polymerase chain reaction) was performed using Phusion HF DNA Polymerase and primers were chosen to identify potato psyllid haplotype and screen for CLso infection. Cytochrome oxidase I (COI) primers confirmed the psyllid's species and haplotype (Crosslin et al. 2011), and Las606/Lss2 and rp0l primers were used to screen the samples for CLso (Table 7; Fujikawa et al. 2012; Mauck et al. 2019; Haapalainen et al. 2018), using the master mix recipe provided in Table 8 and the PCR program provided in Table 9. A positive and negative control were included with each batch of PCR reactions to validate that the assay was working and check for contamination.

Primer name	Primer sequence	Primer reference	Amplicon length
CO1 F3	TACGCCATACTAGCAATCGG	Crosslin et al. 2011	~500 bp
CO1 R3	GAGTAACGTCGTGGTATTCC	Crosslin et al. 2011	
Las606	GGAGAGGTGAGTGGAATTCCGA	Fujikawa et al. 2012	~500 bp
LSS-2	ACCCAACATCTAGATAAAATC	Mauck et al. 2019	
rp01F	CTCTAAGATTTCGGTTGGTT	Haapalainen et al. 2018	637-640 bp
rp01R	TATATCTATCGTTGCACCAG	Haapalainen et al. 2018	

Table 7. The primers that were used to confirm potato psyllids haplotype and CLso.

PCR Reagent	1 reaction (µL)
ddh_2O	10.8
HF x5 Buffer	4
2mm DNTPS	2
Forward Primer	1
Reverse Primer	1
Phusion Polymerase	0.2
Total	19

Table 8. The Phusion HF DNA polymerase PCR mixture per sample.

Order	Temperature (°C)	Time (minutes:seconds)
1	98	5
2	98	00:10
3	60	00:30
4	72	1:00
5	Repeat step 2 40 times	6:40
6	72	10:00
7	10	Infinite

Table 9. The PCR program that was used for all of the JTNP samples.

Gel electrophoresis

PCR products were then mixed with 1µL of loading buffer dye and pipetted into a 1% agarose gel for visualization. The gels were then photographed and annotated after each trial.

Visible amplicons of the expected size were cut out and purified using a Zymoclean™ Gel DNA Recovery kit according to the manufacturer's recommendations.

Sanger sequencing

DNA sequences were sent to Retrogen Inc. or the UCR Genomics Core for Sanger sequencing. The DNA sequence results were manually checked for quality and trimmed using BioEdit sequence alignment editing software (Hall et al., 1999). The majority of the forward and reverse sequences of a sample were checked for agreement and merged to ensure accuracy. Then, the primer sequences were trimmed and BLASTn was used to search the NCBI database for similar sequences.

RESULTS

Potato psyllid sampling

In total, we captured 586 potato psyllids using both our preservative traps and yellow sticky cards. Of these, 536 were collected using the yellow sticky traps, while only 50 were captured in our custom psyllid preservative traps. As illustrated in Figure 10, the number of potato psyllids caught varied depending on the host plant species and trap type. Additionally, there was a notable decline in the quantity of potato psyllids over time, particularly evident in the sticky traps set on *Lycium andersonii.* The host plant, *L. andersonii*, attracted the most psyllids, as evidenced by the number of potato psyllids captured in Figure 11, followed by *Nicotiana obtusifolia, L. cooperi, and P. crassifolia*. A noticeable decline in the number of potato psyllids on *L. andersonii* is depicted in Figure 10. By April 11, 2023, the host plants had begun to senesce or had already senesced, which correlates with the decrease in psyllids found on the passive traps (yellow sticky cards and custom-psyllid preservative traps).

We also identified 2 *Bactericera dorsalis* in the preservative trap on April 11, 2023, at the Cottonwood Spring location on the plant, *Lycium andersonii.*

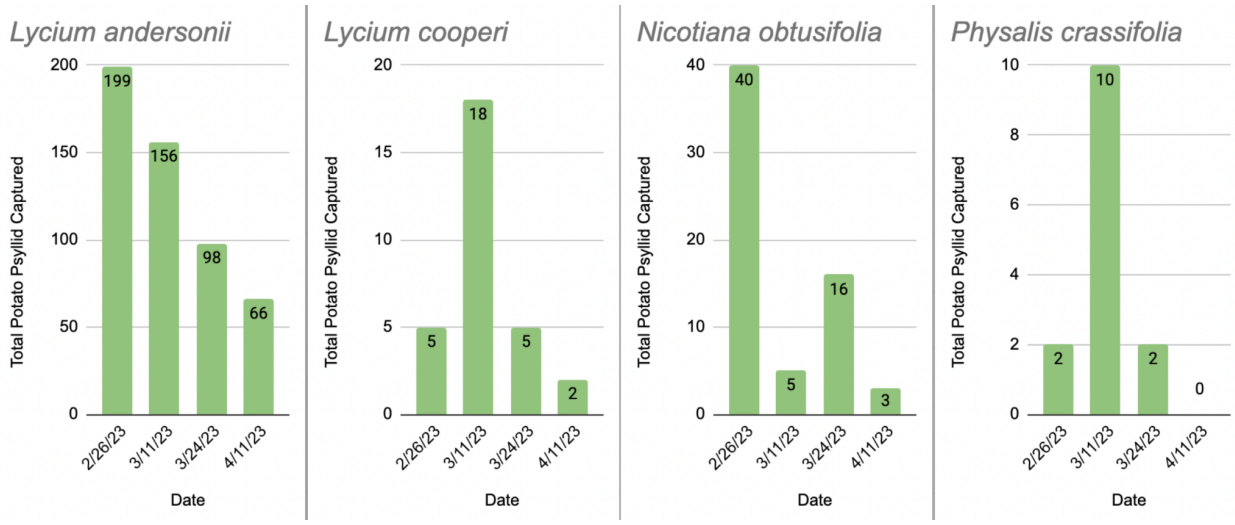

Figure 10. The total amount of *B. cockerelli* captured on yellow sticky cards and preservative traps on each host plant species at each sampling site over the course of the winter-spring growing season. There is a lack of data in some host plant species at a location for every time point because if host plants had completely senesced (when the plant dried up and lost their leaves).

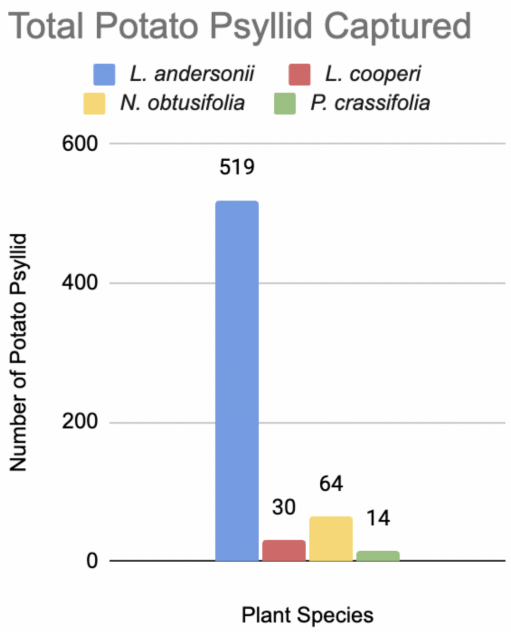

Figure 11. The total number of potato psyllids captured per host plant.

Identification of potato psyllid haplotype and CLso infection status

We extracted DNA from a total of 213 of the potato psyllids we captured on yellow sticky cards throughout the winter-spring 2023 growing season. From the sticky traps, up to 10 specimens per host plant species per sampling location per time point were collected. 208 of the 213 extracted samples had amplicons that showed up in gel electrophoresis and had a working sanger sequence. PCR amplification and Sanger sequencing of the mtCOI gene revealed that all of these specimens were of the Southwestern haplotype with the majority of them having 100% identity match to GenBank accession number KC305359.1 or KC305359.0.

A small subset of samples had one or two single nucleotide polymorphisms compared to the accession number, KC305359.1, as seen in Table 11. This led to samples with a 99% match to the accession number, KC305359.1. None of these 208 potato psyllid specimens tested positive for CLso when screened via PCR with the CLso-specific primer sets rpol-f/-r or Lass606/Lss-2.

SNP position (nt)	GenBank accession number KC305359.1's nucleotide	SNP	Number of samples with SNP	Notes
172	T	C	3	Used only reverse primer for 2 samples. Used forward and reverse primers for 1 sample.
208	G	A	10	Used only reverse primer for the samples.
280	A	G	2	Used only reverse primer for 1 sample. Used forward and reverse primers for 1 sample.

Table 11. Comparing SNPs found in the samples with the GenBank accession number KC305359.1.

DISCUSSION

We performed the first-ever systematic survey for psyllid vectors of the crop pathogen CLso in natural plant communities in the California desert. This work confirmed that the primary North American vector of CLso, the potato psyllid, visits the native solanaceous perennials *Lycium andersonii, Lycium cooperi, Nicotiana obtusifolia,* and *Physalis crassifolia* in this region. It also revealed the presence of an additional potential CLso vector species, *Bactericera dorsalis*, visiting one of the same plant species (*Lycium andersonii*). Ultimately, all of the potato psyllids from all sampling locations and times were found to be of the Southwestern haplotype, and none of these potato psyllids tested positive for CLso.

Our findings that potato psyllids associate with desert plants in the genera *Lycium* and *Physalis* align with the results of studies from other regions, confirming that these two genera are important wild hosts of this vector of CLso throughout its range. For example, in the Pacific Northwest, two introduced species of *Lycium* (*L. barbarum* and *L. chinense*) serve as alternate hosts of potato psyllids when potatoes are not available (Thinakaran et al. 2017), and in Texas, the species *L. carolinianum* and *L. berlandieri* were also recently confirmed to be important native hosts of potato psyllids (Cooper et al., 2022). Similarly, *Physalis longifolia* has been found to host potato psyllids and CLso in the Pacific Northwest (Reyes Corral et al., 2021), and *P. virginiana* serves as a host in eastern Mexico (Delgado-Luna et al., 2023). In JTNP, potato psyllids were also found on the species *Nicotiana obtusifolia*, a genus where potato psyllids have been found before in Nicaragua and Honduras on the species *Nicotiana tabacum* (Munyaneza et al., 2013, Aguilar et al., 2013).

Our discovery of *Bactericera dorsalis,* together with potato psyllids on *Lycium andersonii,* suggests that this species could also potentially transmit CLso among native desert Solanaceae in California. Cooper et al. (2022) recently discovered that *B. dorsalis* regularly associates with and can transmit CLso among wild *Lycium* in Texas. Thus, these findings suggest that *B. dorsalis* warrants further exploration as a potential vector of CLso in California and throughout its range in the southwestern United States. While there are several observations of *B. dorsalis* have been recorded on iNaturalist, there is no peer-reviewed literature that documents *B. dorsalis* being found in California.

Our potato psyllid mtCO1 barcoding results suggest that the potato psyllids found in JTNP are only of the Southwestern haplotype and with no CLso infection. This aligns with previous studies, where the Southwestern haplotype was found in the highest abundance in the desert states of Arizona and New Mexico (Montiel et al., 2016, Workneh, et al., 2018, Swisher et al., 2014.) and in which the Southwestern haplotype has never been implicated as a prominent vector of CLso in crops (Swisher et al., 2014, Rush et al., 2015). This suggests that the Southwestern haplotype may have unique adaptations that allow it to outperform other potato psyllid haplotypes in desert environments and that it may also possess biological differences that make it less efficient as a vector of CLso. However, much more thorough field surveys, as well as controlled CLso acquisition and transmission studies would be needed to confirm this. Currently, the Southwestern haplotype being vectors of CLso has been found in a current non-crop strain, CLso G. According to Kenney et al. (2024), while investigating native perennial hosts such as *Solanum umbelliferum*, there was detection of CLso G within populations of the Southwestern haplotype of potato psyllids. This is significant as it shows that while the Southwestern haplotype may not commonly transmit the agricultural strains of CLso, it is

capable of harboring and potentially spreading other distinct variants like CLso G within native plant communities. However, this does not show that it is possible to carry CLso found in crop plants like potatoes and tomatoes. Moreover, similar to findings by Mauck et al. (2019), where herbarium specimens testing positive for CLso G did not show symptoms of CLso-induced disease conditions typically found in crops, this suggests that the pathogen's effects may differ in non-agricultural environments. It indicates that while the Southwestern haplotype may not be a significant vector in agricultural contexts, its role in natural ecosystems and its interactions with native strains of pathogens could have implications for biodiversity and the stability of native plant communities.

It is important to note that even though we did not detect CLso in the potato psyllid samples tested in this study, this does not rule out the possibility that CLso is present in California desert natural plant communities. CLso may be present in titers that were too low to detect using conventional PCR, or our samples may have been degraded, due to time spent exposed to the elements on yellow sticky cards. Furthermore, CLso infection rates and titers may vary by location, from year to year, and across seasons. Thus, future work will include testing psyllids collected in other seasons and locations, testing less degraded potato psyllid samples (from active collections and preservative traps), and testing host plant tissue directly for CLso infection, possibly using more sensitive methods, such as qPCR.

In conclusion, our results suggest that the native perennial Solanaceae of JTNP are currently at low risk of damage from CLso. However, because the potato psyllid is present and feeding on the host plants, future introductions of CLso into these psyllid populations could pose a threat to the health of natural plant communities in this region. Therefore, continued field

surveys are recommended to ensure early detection of any changes in CLso infection risk in both native plants and in crops in this region.

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