Prototype 3D-Printed Traps Capture *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) Directly into Preservative for Improved Detection of "*Candidatus* Liberibacter solanacearum"¹

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Abstract Potato psyllid, Bactericera cockerelli (Sulc) (Hemiptera: Triozidae), is a key pest of potato (Solanum tuberosum L.) and other solanaceous crops (Solanales: Solanaceae) as a vector of "Candidatus Liberibacter solanacearum" (Lso), the pathogen associated with zebra chip disease of potato. Potato psyllid populations typically are monitored using sticky card traps, and psyllids collected from sticky traps often are subjected to polymerase chain reaction (PCR) to monitor the incidence of Lso within psyllid populations. Psyllids collected from sticky traps are often mangled, desiccated, and coated with sticky residue, which may interfere with detection of Lso by PCR. A recently developed prototype 3-dimensionalprinted trap that captures insects directly into a preservative (70% ethanol) was previously tested for monitoring psyllid populations. The capture of psyllids directly into a preservative may reduce degradation of DNA or protect specimens from PCR-inhibiting contaminants, thus improving the detection of Lso by PCR. Our goal was to compare the detectability of Lso in psyllids captured into preservative (prototype trap) to that in psyllids removed from sticky card traps. Overall, detection rates were higher in psyllids from the prototype trap than from sticky card traps. This improvement in Lso detection appeared to be partly due to the specimens yielding more DNA of higher quality. Results of this study demonstrate that compared with sticky card traps, a trap that captures psyllids directly into a preservative provides higher quality specimens for collection of molecular data, including pathogen diagnosis, population genetics, and molecular gut content analysis.

Key Words potato psyllid, *Bactericera cockerelli*, Liberibacter, zebra chip, monitoring, 3D-printed prototype

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The potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is a major pest of potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) as a vector of the plant pathogen "*Candidatus* Liberibacter solanacearum" (Lso) (="*Ca.* L. psyllarous") (Munyaneza 2012). This pathogen is associated with foliar dieback, reduced yield in infected crops, and the production of striped patterns, which is referred to as zebra chip disease in infected potato tubers. There are currently no methods to directly control Lso, so management consists of repeated applications of insecticides to reduce populations of the psyllid vector. A major challenge to psyllid and zebra chip management is the inability to predict the timing and severity of psyllid infestations and the likelihood that the psyllids are carrying the pathogen.

Potato psyllid populations are monitored with yellow sticky card traps in the potato-growing regions of the western United States (Wohleb and Waters 2014; Wohleb 2017; Johnson et al. 2017; Wenninger et al. 2017). Results of psyllid areawide monitoring programs are used by growers and crop consultants to estimate when psyllids are colonizing fields of potato and to time insecticide applications to reduce the risk of zebra chip (Crowder and Wohleb 2017). Psyllids captured on sticky card traps often are subjected to polymerase chain reaction (PCR) to assess Lso infection rates within psyllid populations (Goolsby et al. 2012; Wenninger et al. 2013, 2017; Dahan et al. 2017; Johnson et al. 2017; Wohleb 2017). Specimens captured from sticky traps are frequently mangled, desiccated, and covered with sticky residue and wind-blown contaminants, which are likely to impact the ability to detect Lso with PCR. Indeed, a recent study using gut content analysis to track landscape movement of psyllids reported greater difficulty in amplifying plant DNA from psyllids captured on sticky traps than from specimens collected directly from plants (Cooper et al. 2019).

A potential alternative to sticky traps is a prototype 3-dimensional (3D)-printed trap designed to capture psyllids directly into preservative (Horton et al. 2019) (Fig. 1A). This prototype trap was developed by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, for monitoring Asian citrus psyllid, Diaphorina citri Kuwayama (Hemiptera: Liviidae). The prototype trap takes advantage of psyllid behavior, including their attraction to yellow and tendency to walk upward toward light (Adams et al. 1983; Krysan and Horton 1991; Hall 2009). Horton et al. (2019) reported that the prototype trap is not as efficient at capturing psyllids as sticky card traps but that samples recovered from the prototype trap are easier to process than those recovered from sticky cards, in part, because the trap captures fewer nontarget insects and because there is no sticky residue. The capture of specimens directly into preservative rather than on sticky cards may slow DNA degradation and protect specimens from contamination by PCR inhibitors and, therefore, provide specimens of higher quality for pathogen detection and molecular gut content analysis. The objective of our study was to compare the relative abilities to detect Lso from psyllids captured on sticky card traps or the prototype psyllid trap.

Materials and Methods

Potato psyllids and psyllid traps. Potato psyllids used in each of our assays were obtained from an Lso-infected colony maintained at 22°C with a 16:8-h



Fig. 1. A prototype 3D-printed trap designed to capture psyllids and other small insects directly into a preservative (A). Potato psyllids placed and marked on a yellow sticky card (B). Traps were suspended from a line in the first experiment (C) and were hung from wood stakes or shepherd hooks in the second experiment (D).

photoperiod on the potato cultivar "Ranger Russet." The colonies were started from psyllids originally collected in Prosser, WA, during the summer of 2016 and were identified as western haplotype based on nucleotide sequences of cytochrome oxidase 1 (Swisher et al. 2012). The colony was periodically checked for Lso infection rates using PCR to amplify and detect a region of 16S of Lso using primers AO2/Ol2c (Crosslin et al. 2011).

The prototype 3D-printed trap used in our experiments was previously described by Horton et al. (2019). The traps were constructed from yellow plastic by using a MakerBot Replicator 2 printer (MakerBot Industries, Brooklyn, NY) (Fig. 1A) (Howe et al. 2017; Snyder et al. 2017). The standard yellow sticky card (13×18 cm; Alpha Scents Inc., West Lin, OR) being used in current monitoring programs for potato psyllid was included for comparison with the prototype 3D trap (Fig. 1B).

Trap comparisons. Two independent field trials were conducted at the USDA laboratory near Wapato, WA. The experimental setups for both trials were nearly identical, with the exception for methods of hanging traps and the duration the traps were maintained outside. In both trials, 20 Lso-infected psyllids per trap were anesthetized with CO₂ and placed directly into the preservative-filled culture tube attached to the prototype trap (n=5) or were scattered directly onto the sticky card traps (n = 5). The locations of psyllids on sticky card traps were marked to ensure collection of colony insects at the end of the trial (Fig. 1B). In the first trial, the traps were suspended from a cord between two trees (Fig. 1C). This trial was performed during a warm period in late March with high temperatures averaging 13.6°C and low temperatures averaging 0.5°C (http://weather.wsu.edu; Konnowac Pass weather station). We intended to collect the psyllids after 7 d, but the assay was terminated after only 3 d due to the disappearance of many psyllids from the sticky cards. In the second trial, performed in late April of 2018, high temperatures averaged 23°C and low temperatures averaged 4°C (http://weather.wsu.edu; Konnowac Pass weather station), prototype traps were suspended from shepherd hooks and sticky card traps were stapled to wooden stakes at a height of about 1 m (Fig. 1D). The sticky card traps captured substantial numbers of nontarget insects during the second trial, and most of the potato psyllids placed on the traps were recovered after 7 d. In both trials, psyllids were assayed only if the white patterns on the thorax characteristic of potato psyllid were clearly visible, following previous observations of coauthors C. Wohleb and T. Waters who regularly use sticky cards to capture psyllids to test for the presence of Lso-infected psyllids. For controls, psyllids were obtained directly from the same Lso-infected colony and immediately stored at -80°C to be paired with trap sets. All psyllids were stored in -80°C until the molecular analyses could be completed. Wild or cultivated hosts of potato psyllid were not located near the traps, reducing colonization of traps by noncultured psyllids.

Molecular and statistical analyses. DNA was purified from each psyllid by using the cetyltrimethylammonium bromide precipitation method (Zhang et al. 1998; Crosslin et al. 2011) and then suspended in 50 μ l of nuclease-free water. The quality and quantity of DNA in each sample were estimated using a Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, MA).

The presence or absence of Lso was determined using conventional PCR with primers OA2/OI2c to amplify the 16S ribosomal RNA region of the pathogen. Each 20- μ I PCR reaction included 10 μ I of PCR master mix (Amplitaq Gold 360 PCR Master Mix; Applied Biosystems, Foster City, CA), 7 μ I nuclease-free water, 1 μ I of each primer (250-nM final concentration), and 1 μ I of DNA sample. PCR conditions included 94°C for 5 min, then 35 thermal cycles (94°C for 30 s, 66°C for 30 s, and 72°C for 1 min), followed by 10 min at 72°C. The 1,168-bp amplicons were observed on a 1% agarose gel with ethidium bromide.

Degradation of DNA and the presence of impurities were compared among treatments by observing DNA on a 0.5% agarose gel stained with ethidium bromide. The quantity of DNA loaded onto gels varied among replications between 50 ng and 740 ng (mean of 285 ng) but was standardized among treatments within replications based on the sample with the lowest amount of DNA.

Statistical analyses were done using the GLIMMIX procedure of SAS (SAS Institute 2013). The two field trials were analyzed separately. Lso detection rates



Fig. 2. Psyllids were removed from traps, presumably by a predator, in the first experiment when very few nontarget insects were captured.

(Lso positive/total number of specimens) were compared among treatments (control, prototype trap, and sticky card trap) by logistic regression. Quantity of DNA (ng/µl) was compared among treatments by analysis of variance. In both analyses, treatment was included as a fixed effect and replicate (each pair of traps and a corresponding group of insects used as control) was included as a random variable. Differences among means were determined using the ADJUST = SIMULATE option of the LSMEANS statement when the overall analysis of variance.

Results and Discussion

Both field trials were performed well away from cultivated or wild hosts of the potato psyllid to minimize opportunities for field-originating potato psyllids to colonize traps. Thus, we are confident that all specimens processed for PCR had indeed originated from our Lso-infected colony, which had nearly 100% infection rate during these trials. One unexpected finding was the removal of psyllids from the sticky card traps. This result is likely due to scavenging birds or insects (Fig. 2). This phenomenon has previously been observed on sticky card traps deployed in commercial potato fields (Wohleb and Waters, unpubl. data). A larger percentage of psyllids remained on the traps during the second trial when more nontarget insects were captured, possibly because predators preferred the larger insects to the psyllids.

Rates of Lso detection differed among treatments in both trials (Trial 1: F = 8.5; df = 2, 8; P = 0.010; Trial 2: F = 11.5; df = 2, 8; P = 0.005). The results of both trials indicated that detectability of Lso in psyllids was substantially reduced in specimens from sticky card traps relative to specimens from the prototype 3D-printed trap or specimens directly from the colony (Fig. 3). Detection rates in the second trial were



Fig. 3. Incidence of Liberibacter detection by PCR from psyllids collected directly from an infected colony, maintained in the 3D-printed prototype psyllid trap, or maintained on sticky card traps for 3 d (A) or 1 week (B). Different letters denote significant differences among treatments ($\alpha = 0.05$).

statistically lower among psyllids from the prototype trap than from psyllids collected directly from the colony. In Trial 2, traps had been deployed for 7 d, in contrast to the 3-d period in the first trial (Fig. 3). Overall, results demonstrated a diminished ability to detect Lso in known-infected psyllids, regardless of trap type, but also showed that detectability was higher using the prototype trap instead of sticky cards.

The amount of DNA that we were able to extract from specimens differed significantly among treatments in both trials (Trial 1: F = 31.7; df = 2, 8; P < 0.001; Trial 2: F = 31.7; df = 2, 8; P < 0.001). The two experiments were consistent in showing that DNA was extracted at higher amounts in specimens from the prototype trap than specimens from sticky card traps (Fig. 4). However, the amount of DNA extracted from psyllids collected from either trap was reduced compared to amounts in psyllids obtained directly from the colony (Fig. 4). Psyllids removed from sticky card traps were often in poor condition and coated in sticky residue, which likely interfered with DNA extraction.

The ratio of absorbance at 260 nm and 280 nm was about 1.8 for nearly all samples, indicating that samples in all treatments were mostly void of protein or other contaminants that absorb at 280 nm (Thermo Scientific 2009). Furthermore, the 260 nm/230 nm ratio of absorbance generally fell between 2.0 and 2.2 for most samples indicating that samples were mostly void of carbohydrate or phenol contaminants which absorb at 230 nm (Thermo Scientific 2009). Degradation of DNA and the presence of impurities that may not absorb at 280 or 220 nm were assessed by observing genomic DNA on agarose gels. Although genomic DNA was visible from most samples collected directly from the colony, genomic DNA was rarely visible from samples collected from sticky card traps, indicating substantial degradation of DNA from these specimens (Fig. 5). DNA degradation in specimens from the prototype trap appeared intermediate to specimens directly from colony and specimens from sticky traps, but degradation appeared more severe in the second experiment when



Fig. 4. Quantity of DNA extracted from psyllids collected directly from an infected colony, maintained in a 3D-printed prototype psyllid trap, or maintained on sticky card traps for 3 d (A) or 1 week (B). Different letters denote significant differences among treatments ($\alpha = 0.05$).

traps were deployed for a full week. The gels also indicated that most DNA samples from the sticky card specimens had contaminants that may interfere with PCR (Fig. 5). Overall, these observations indicate that DNA from psyllids maintained on sticky card traps is usually degraded and contaminated with impurities and that the use of the prototype trap improves the quality of sample DNA.

Results of our study demonstrated that the capture of psyllids on sticky card traps reduces the quality of the specimens for PCR detection of Lso. These findings are consistent with molecular gut content studies showing a reduced ability to amplify plant DNA from psyllids captured on sticky card traps versus those collected directly from plants (Cooper et al. 2019). We speculate that poor rates of Lso



Fig. 5. Representative gel to observe DNA degradation and impurities in samples from psyllids maintained on sticky card traps (S), in a prototype trap with 70% ethanol as a preservative (P), or collected directly from a laboratory colony (C). DNA quantity varied among replications but was standardized among treatments within replications grouped on the gel.

detection from psyllids collected from sticky card traps are due to combined factors, including the desiccated and fragmented condition of the psyllids, DNA degradation from environmental exposure, and the presence of PCR inhibitors including windblown dust and sticky trap residue. A preliminary study performed in a greenhouse did not indicate statistically significant differences between trap types in the ability to detect Lso from psyllids (Wentz et al. unpubl. data), suggesting that field conditions may exacerbate factors contributing to the inability to detect Lso in specimens from sticky cards. Since the 2011 outbreak of zebra chip disease in the Pacific Northwest, psyllid monitoring programs using sticky card traps have suggested a very low rate of Lso infection in psyllid populations. These low infection rates are consistent with the low rate of zebra chip disease in harvested potatoes since the 2012 growing season. However, our results suggest that Lso may be more prevalent in psyllid populations than has been indicated by PCR results derived from specimens captured in monitoring programs using sticky cards. Bactericera cockerelli captured in traps often are used to collect molecular data, including the presence or absence of Lso, genetic haplotype (Swisher et al. 2012), and dietary history of the specimens (Cooper et al. 2019). Although the prototype trap is less efficient at capturing psyllids than the sticky card trap, the capture of specimens directly into a preservative provides an alternative means of capturing psyllids for molecular studies.

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