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Drought stress and pathogen infection alter feeding behavior of a phytopathogen vector

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Abstract

The impact of drought stress on tripartite plant-pathogen-vector interactions constitutes a complex and largely understudied field of plant-insect interaction. A number of studies explored these topics using aphid vectors of plant pathogens, but few have considered the interactions between drought-stressed plants and pathogen-transmitting psyllids. The potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), is one of the key pests of solanaceous crops in the USA that causes direct injury as well as indirect injury through transmission of a bacterial pathogen, *Candidatus* Liberibacter solanacearum (Lso), the causal agent of zebra chip. Previous studies explored the impact of Lso infection and drought stress on *B. cockerelli* development and reproductive rate separately, but no research to date has evaluated whether drought stress and Lso infection alter feeding behavior of the insects. We explored this using the electrical penetration graph (EPG) technique and monitored feeding behavior of Lso-infected and uninfected potato psyllids on well-watered and drought-stressed tomato (*Solanum lycopersicum* L., Solanaceae). We found that drought stress had a significant effect on feeding behavior associated with salivation into the phloem and phloem ingestion, both linked to Lso transmission. Furthermore, infected potato psyllids in particular produced a higher number of events associated with these feeding behaviors and remained in these phases longer in well-watered plants than in plants that were under drought stress. We also reported a new and previously undescribed waveform H of unknown biological function that was produced by the psyllids. This is the first study that considered the impact of bacterial infection and concomitant drought stress on feeding behavior of an insect quantified using EPG.

Introduction

It is well established that drought stress mediates two-way interactions between plants and herbivores (Gutbrodt et al., 2011; Johnson et al., 2011; Tariq et al., 2012; Szczepaniec & Finke, 2019), plants and pathogens (Boyer, 1995; Thaler & Bostock, 2004; Thompson et al., 2013), and tripartite plant-pathogen-vector interactions (Szczepaniec & Finke, 2019). The effects of drought on aphid performance can be positive (Khan et al., 2010; Mewis et al., 2012), negative (Mcvean & Dixon, 2001; Hale et al., 2003), or neutral (Salas & Corcuera, 1991; Pons & Tatchell, 1995). Drought stress can also have variable consequences for plant-pathogen interactions (Mauch-Mani & Mauch, 2005; Fujita et al., 2006; Asselbergh et al., 2008) that are likely to be species specific. For instance, drought stress increased the development of Pierce's disease symptoms caused by the bacterial pathogen *Xylella fastidiosa* in grapevines (Thorne et al., 2006).

Furthermore, there is accumulating evidence that drought stress has complex, frequently unique consequences for plant-vector-pathogen interactions (Szczepaniec & Finke, 2019). For example, Krugner & Backus (2014) showed that drought stress reduced the frequency of probes by the glassy winged sharpshooter, *Homalodisca vitripennis* Germar, which are critical for transmission and

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spread of *X. fastidiosa*. The authors of this study did not quantify the consequences of drought stress for dispersion of *H. vitripennis*, which in other systems has been correlated with increased dispersal and increased risk of transmission. For example, drought stress enhanced dispersal of bird cherry-oat aphids, *Rhopalosiphum padi* L., and increased the proportion of plants infected with *Barley yellow dwarf virus* (BYDV) (Smyrnioudis et al., 2000). Moreover, densities of *R. padi* increased at a faster rate on drought-stressed BYDV-infected plants than on drought-stressed non-infected plants (Smyrnioudis et al., 2000; Davis et al., 2015) suggesting that the interaction between virus infection and water availability can alter the quality of host plants. These outcomes suggest that drought stress and concomitant pathogen infection alter quality of the plants, thereby promoting pathogen fitness through improved fitness of the vector. These interactions have been explored in other aphid species and plants as well. For example, drought stress enhanced the transmission rate of *Cauliflower mosaic virus* and *Turnip mosaic virus* transmitted by an aphid to *Brassica rapa* L. (van Munster et al., 2017). Furthermore, Nachappa et al. (2016) showed that viruliferous soybean aphids, *Aphis glycines* Matsumura, took a longer time to probe on drought-stressed plants than on well-watered plants, which likely resulted in lower virus infection illustrated by fewer viral RNA copies of *Soybean mosaic virus* in drought-stressed plants.

Few studies have considered the interactions between drought-stressed plants and pathogen-transmitting psyllids. In one study, drought stress increased survival and population growth rate of potato psyllids, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), infected with the bacterium *Candidatus* Liberibacter solanacearum (Lso) by 60% (Huot & Tamborindeguy, 2017). Drought stress also reduced emission of volatiles induced by another bacterial pathogen, *Candidatus* Liberibacter asiaticus, which decreased the attraction to infected plants of its vector, Asian citrus psyllid, *Diaphorina citri* Kuwayama. It also lowered the recruitment of the parasitoid *Tamarixia radiata* (Waterston) to plants infested with Asian citrus psyllid and decreased its biological control (Martini & Stelinski, 2017).

The potato psyllid is one of the key pests of solanaceous crops in the USA (Prager & Trumble, 2018). The insect can transmit Lso, the causal agent of zebra chip (Munyaneza et al., 2007), that has contributed to severe economic losses for potato production in the USA (Greenway & Rondon, 2018). Notably, Lso has been shown to reduce *B. cockerelli* fecundity and nymph survival on tomato (Nachappa et al., 2012), and in combination with drought stress the pathogen can alter potato psyllid survival as well (Huot & Tamborindeguy, 2017). Whereas these studies highlight the impact of Lso infection and drought stress on *B. cockerelli* performance separately, it is unknown how drought stress alters feeding behavior of the insects, thereby affecting Lso transmissibility.

In the current study, we tested the hypothesis that drought stress and Lso infection alter feeding behavior of *B. cockerelli* on tomato. We used the electrical penetration graph (EPG) technique and monitored feeding behavior of Lso-infected and uninfected potato psyllids on well-watered and drought-stressed tomato, *Solanum lycopersicum* L. (Solanaceae). This is the first study that quantified the impact of bacterial infection and drought stress on feeding behavior of an insect using EPG. This work contributes to the increasing body of research focused on the simultaneous consequences of drought stress and pathogen infection on the biology of insect vectors.

Materials and methods

Insects

Colonies of Lso-free and -infected potato psyllids were established from naturally occurring infestations of potato psyllids in the Panhandle of Texas and were maintained at the Texas A&M AgriLife Research greenhouse complex, located at the Plant Stress Laboratory in Bushland, TX, USA, for 1 year prior to experiments. All psyllids were 'Central USA haplotype', which is one of four genetically distinct populations of *B. cockerelli* identified thus far (Swisher et al., 2012). All psyllids were maintained on tomato (cv. Lance) in insect-proof mesh cages (60 × 60 × 60 cm; MegaView Science Education Services, Taipei, Taiwan). Colonies were maintained in the greenhouse at 21 ± 3 °C (night) to 30 ± 3 °C (day). In order to test for Lso infection in the psyllids, DNA from individual insects was extracted using QuickExtract Plant DNA Extraction Solution (Epicentre, Madison, WI, USA) and used for diagnostic real-time PCR to verify the presence or absence, and the haplotype of bacterium following previously described methods (Brownlie et al., 2009; Li et al., 2009). All reactions were performed on ViiA 7 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The presence of Lso in psyllids was detected using an absolute quantification method following Workneh et al. (2018). The Lso haplotype assay was also performed on the same machine following Workneh et al. (2018). A comparative cycle threshold method was used to quantify Lso '*Ca*. L. solanacearum' relative to a calibrator and an endogenous 18S RNA control. The reaction mix consisted of TaqMan Universal Master Mix (Applied Biosystems), 0.3 μM forward primer LsoF (Brownlie et al., 2009), 0.3 μM reverse primer HLBr (Li et al., 2006), and 0.25 μM HLBp TaqMan probe (Li et al., 2006). Eukaryotic 18S

rRNA (VIC/MGB probe, primer limited; Applied Biosystems) was used as the endogenous control. All psyllids used in experiments that were infected with the bacterium carried Lso haplotype B. Absence of the pathogen was confirmed in the uninfected psyllids using the same methods.

Plants

Tomato plants (cv. Moneymaker) for the experiments were grown in environmental chambers in 15-cm-diameter (6-inch) pots (Hummert International, Earth City, MO, USA) with 830 soilless media (Mastermix, Quakertown, PA, USA). All plants were maintained at 60–70% r.h., 24 ± 1 °C, and L16:D8 photoperiod. Plants were watered 3× per week ad libitum and received Miracle Gro solution (Scott's Company, Marysville, OH, USA) as per label instructions once per week.

Water-stress treatments

Plant water-stress treatments were established according to Nachappa et al. (2016). Briefly, 500 g of Mastermix 830 soilless media was weighed, and fully saturated with water in 15-cm pots. Saturated medium was weighed and a Waterscout SM100 soil moisture probe (Spectrum Technologies, Aurora, IL, USA) was used to determine volumetric water content (% VWC). The saturated medium was weighed every day and allowed to air dry until all moisture was lost and weight of the media did not change over a course of 3 days. Volumetric water content and weight of water loss were monitored daily. A calibration curve based on average VWC and corresponding mass of water was computed, based on which the volume of water to be used to maintain each drought stress treatment was determined. Three-week-old tomato plants were exposed to two water-stress treatments: drought stress (25% of field capacity or FC corresponding to 7.6% VWC) and well watered (75% FC, corresponding to 17.9% VWC). The plants were maintained at these conditions for 3 days prior to the start of the EPG experiments. The water-stress conditions were maintained by measuring the soil water content daily and the appropriate amount of water was applied as needed to maintain soil water content at 25 or 75% FC. As a second measure of the efficacy of drought stress treatment, leaf water potential (LWP) was measured on the 3rd day of the treatment. In drought-stressed plants, on average LWP was −0.87 ± 0.05 MPa and in well-watered plants LWP was −0.38 ± 0.01 MPa.

Electrical penetration graph recordings

The feeding behaviors of Lso-free and Lso-infected potato psyllid were recorded using the EPG on a GIGA-8 DC-EPG system (EPG Systems, Wageningen, The Netherlands) (Tjallingii & Esch, 1993) with a 1 giga ohm input resistance. Output from the EPG was recorded using the Stylet+d software and analyzed using the Stylet+a software (v.01.30; both from EPG Systems). Three-week-old whole plants were used for EPG recordings. Wired psyllids were placed on the abaxial surface of the uppermost fully expanded leaf and allowed to feed for 12 h under ambient laboratory conditions. The plant electrode was inserted into the soil at the base of the root and voltage was adjusted to 30 mV. Adjustments were made in the initial 20 min so that the output would fit in the +5 to −5 V window within the Stylet+d software (EPG Systems). Wiring of post-teneral adult psyllids was carried out according to Nalam et al. (2018) with a few modifications. Adult psyllids collected from Lso-free and -infected colonies were starved for 1 h prior to wiring in separate Petri dishes. Adult psyllids were attached to the insect electrode using a gold wire (2 cm long, 18 µm diameter) and silver glue (EPG Systems). Each psyllid was allowed to access tomato leaves and feeding was monitored for 12 h. The recordings were replicated using a different set of psyllids and plants for each of the treatment combinations (water-stress and psyllid infection status) until a minimum of 18 replicates for each treatment combination were obtained to compensate for behavior variability among the individual psyllids.

Each feeding experiment was analyzed and waveforms were coded using Stylet+a software to determine the amount of time spent by the psyllid in the four main phases (Butler et al., 2012). A phase is defined based on the location of the psyllid stylet either outside or inside the plants tissue and the activities in which the psyllid is engaged. Phase 1 is the non-probing (NP) phase, when the stylet has not yet penetrated plant tissue. Phase 2 is pathway C or probing phase when the psyllid stylet has entered plant tissue and is actively attempting to find either the phloem or xylem tissue. Phase 3 is the sieve element phase, which includes three sub-phases. These include a phase during which the stylet has made initial contact with phloem (D), begins salivating into the sieve elements (E1), and is actively ingesting phloem sap (E2). Finally, phase 4 is the xylem phase (G) when the insect is passively ingesting xylem sap. Additionally, variables that quantified probing behavior were also measured. The quantification of the feeding behaviors of the psyllids was performed using a Microsoft Excel Workbook (v.4.4.3) that allows for the automatic variable calculation of EPG data (Sarria et al., 2009) with the following modifications to account for the H and D waveforms that occur in psyllids but not in aphids. The H waveform was coded as 6 in Stylet+a and matches the F waveform found in aphids. Waveform D that occurs before E1 was coded as 8 in Stylet+a and corresponds to the potential drop (pd) that is present during

aphid feeding. To calculate total time spent in C, the time spent in 8 or pd was subtracted from the value for the total time spent in C derived from the Excel Workbook (Sarria et al., 2009). Finally, the time spent in pd was added to total time spent in E1 and E2 to obtain the total time spent in E.

The number of transitional events for each waveform were determined to construct a behavioral kinetogram as per Ebert et al. (2018). The area of the circles in the kinetogram represents the time spent in each feeding behavior. The arrows were produced by dividing the transitional events for each waveform by the total number of transitional events. As the values for both circles and arrows represent single, summed values of each treatment, statistical analyses is not possible.

Statistical analysis

The experiment was conducted as a 2 × 2 factorial randomized complete design, with two water status treatments (drought- and well watered) and two bacterial infection treatments (Lso-free and -infected). We based our analyses on Ebert v.2.0 analysis program (http://www.crec.ifas.ufl.edu/extension/epg/sas.shtml) that uses the SAS software program. Numbers of infected and uninfected psyllids generating each waveform were compared within each water treatment using t-tests. To analyze the effects of water and infection on feeding behavior of the psyllids we performed a mixed model ANOVA using Proc GLIMMIX. Prior to analyses, the EPG data for durations were log-transformed, frequency of behaviors was $\sqrt{x}$-transformed, and percentages was arcsine transformed.

Results

Psyllid feeding waveforms

The psyllids produced the distinct waveforms described previously for *B. cockerelli*. These included the non-probing phase (baseline voltage, NP); the pathway phase or intercellular stylet penetration (C); the sieve element phase (E), which includes initial contact with phloem tissue (D); salivation into phloem sieve elements (E1); phloem sap ingestion (E2); and ingestion of xylem sap (G). We also noted a waveform that has not been previously reported for potato psyllids, which we have labelled H, which shows a relatively low amplitude (5–15%), extracellular voltage level, and frequency ranges of 4–6.2 Hz (Figure 1). Waveform H tended to appear during the pathway phase and was distinct from waveforms A and B, which together comprise the pathway phase as proposed by Cen et al. (2012). The behavioral kinetogram (Figure 2) indicates the possible transitions from and to each waveform. Non-probing always transitions to C. From C, the psyllid can transition back to NP, G, or D or in certain cases to waveform H. From G and H, the transition always occurs to C. The most common transition from D occurs to E1. From E1, transitions to C and E2 can occur. Similarly, from E2 the psyllids can transition to E1 and C. The number of potato psyllids that produced each waveform did not differ

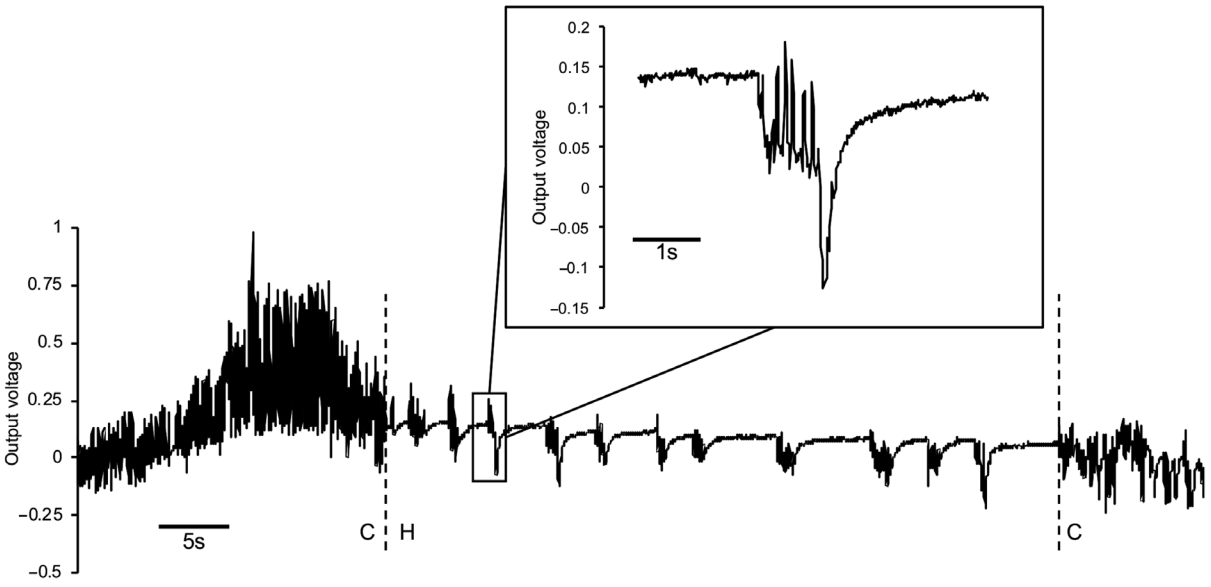

Figure 1 Waveforms C and H observed in electrical penetration graph (EPG) recordings of Lso-free and -infected potato psyllids on leaves of tomato plants exposed to drought stress. The inset shows the expanded view of the basal repetitive pattern that characterizes waveform H.

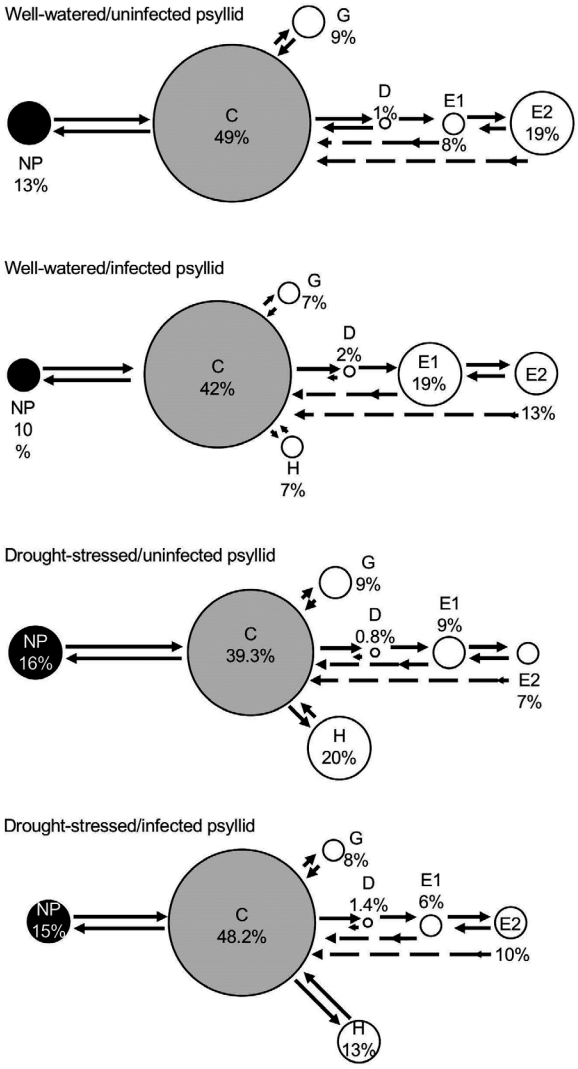

Figure 2 *Behavioral kinetogram of the Lso-free and -infected potato psyllid on leaves of well-watered and drought-stressed tomato plants. Circle areas represent the proportion of time spent by the psyllid in different behaviors (NP, C, D, E1, E2, G, and/or H; see Material and methods for their description). Arrows represent transitions with arrow length proportional to frequency. Dashed arrows are included to make transitions clear.*

among treatments, with one exception – significantly more Lso-infected *B. cockerelli* feeding upon well-watered plants produced the E2 waveform compared to the uninfected psyllids on the plants that received ample irrigation (Table 1).

Impact of drought stress on psyllid probing behavior

The interaction between the water status of the plants and infection with Lso had significant effects on the probing behavior of *B. cockerelli* (Table 2 and Table S1). It is noteworthy that the time to first D as well as the total duration of the sieve element phase (duration of E1 and E2) were significantly affected by the interactive effect of the water and infection treatments (Table 2 and Table S1).

Impact of drought stress and Lso infection on psyllid salivation behavior

Drought stress and infection status of the psyllids had an interactive effect on the number of waveforms E1 (i.e., salivation into sieve elements) ($F_{1,70} = 15.7$) and their duration ($F_{1,70} = 33.1$, both $P<0.001$) (Figure 3). Potato psyllids infected with Lso and feeding on well-watered plants generated 2.5× as many of these waveforms as uninfected psyllids exposed to plants under the same water treatment and infected psyllids on drought-stressed tomato (Figure 3A). The time spent in salivation (E1) was also the highest for Lso-carrying psyllids on well-watered plants and lasted 2× longer compared with uninfected psyllids exposed to well-watered plants (Figure 3B). There were no significant effects of the water treatment on the number ($F_{1,70} = 0.94$, $P = 0.34$) or duration ($F_{1,70} = 3.62$, $P = 0.061$) of E1 waveforms, but the infection status affected both the number ($F_{1,70} = 9.25$, $P = 0.003$) and duration ($F_{1,70} = 13.1$, $P<0.001$) of these waves. These results illustrate an interaction between drought stress and Lso infection on salivation by the psyllids.

Impact of drought stress and Lso infection on psyllid phloem ingestion behavior

Similarly, we noted interactive effects of water and infection on the number ($F_{1,58} = 4.99$, $P = 0.029$) and duration ($F_{1,58} = 8766.14$, $P<0.001$) of the ingestion phase (waveform E2) (Figure 4). The mean numbers of these waveforms generated by infected and uninfected psyllids exposed to either water treatments were comparable (Figure 4A). On the other hand, Lso-infected psyllids feeding on the well-watered tomato spent over 6 h ingesting the phloem sap, whereas the uninfected psyllids generated E2 waveforms for approximately 3 h (Figure 4B).

Furthermore, the time psyllids spent in the sieve element phase (salivation and ingestion, E1 + E2) was affected by the interaction between the water treatment and Lso infection ($F_{1,56} = 39355.5$, $P<0.001$; Figure 5). Infected psyllids exposed to the well-watered plants spent over 35% of time in the sieve element phase, compared to the Lso-infected psyllids feeding on drought-stressed tomato that spent only about 15% of time in this phase. Taken together, it appears that drought stress had a greater impact on the sieve element phase than the Lso infection.

Table 1 Number of uninfected and Lso-infected potato psyllids that did (+) or did not (−) produce waveform types NP, C, D, E1, E2, G, and/or H (see Material and methods for their description) on well-watered (control) or drought-stressed tomato plants

			NP			C			D			E1			E2			G			H		
Water	Infection	n	+	−	P[1]	+	−	P	+	−	P	+	−	P	+	−	P	+	−	P	+	−	P
Well-watered	Uninfected	19	18	1	1	19	0	nt[2]	17	2	1	17	1	nt	14	5	0.05	15	4	0.66	0	19	0.22
	Infected	18	18	0		18	0		17	1		17	1		17	1		16	2		2	16	
Drought-stressed	Uninfected	22	22	0	nt	22	0	nt	18	4	0.7	17	5	0.54	14	8	0.7	20	2	1	4	18	0.41
	Infected	27	27	0		27	0		23	4		22	5		16	11		25	2		10	17	

n = sample size.
[1]P = P-value based on t-tests comparing means within a water treatment.
[2]nt = not tested (no events were recorded, or no difference between treatments).

Impact of drought stress and Lso infection on other probing behaviors

Lastly, we noted lack of significant differences in the number of the remaining feeding behaviors. Specifically, there were no significant differences in the number of NP, C, D, and G phases, and comparable numbers of each of these were produced by the psyllids regardless of treatment (Table 3). Duration of these behaviors, however, was significantly affected by the interaction between the water status of the plants and the infection (Table 4). The time the psyllids spent in each of these phases was also comparable across the treatments for the non-probing phase (water*infection: $F_{1,82} = 0.3$, $P = 0.86$; water: $F_{1,82} = 1.15$, $P = 0.29$; infection: $F_{1,82} = 0.52$, $P = 0.47$), pathway phase (water*infection: $F_{1,82} = 2.39$, $P = 0.13$; water: $F_{1,82} = 0.74$, $P = 0.39$; infection: $F_{1,82} = 0.15$, $P = 0.70$), phloem contact (water*infection: $F_{1,82} = 0.12$, $P = 0.73$; water: $F_{1,82} = 1.64$, $P = 0.13$; infection: $F_{1,82} = 0.11$, $P = 0.75$), and xylem phase (water*infection: $F_{1,82} = 0.8$, $P = 0.82$; water: $F_{1,82} = 1.18$, $P = 0.28$; infection: $F_{1,82} = 0.92$, $P = 0.34$).

Discussion

Drought stress has been shown to exert significant impacts on plant physiology (Asselbergh et al., 2008) that have profound implications for phloem-feeding insects in general (Huberty & Denno, 2004) and important, yet poorly understood, consequences for insect vectors of plant pathogens in particular (Szczepaniec & Finke, 2019). In the current study, we investigated the effects of drought and pathogen infection on feeding behaviors of a psyllid vector. Our results demonstrate that drought and pathogen infection have varying consequences on potato psyllid feeding behavior in ways that can impact disease transmission.

Only a handful of studies have considered the interaction between drought stress and pathogen infection on feeding behaviors of a phytopathogen vector. Krugner & Backus (2014) showed that drought stress reduced the frequency of probing behavior of a xylem feeder, which can potentially impact transmission of *X. fastidiosa*, the bacterial pathogen of Pierce's diseases in grapes. Similarly, Nachappa et al. (2016) found that viruliferous aphids took a longer time to generate the initial potential drop on drought-stressed soybean plants and the number of intracellular punctures was the lowest on drought stress plants compared to well-watered plants. Furthermore, these authors also confirmed that drought-stressed soybean plants harbored lower *Soybean mosaic virus* infection compared to well-watered plants. The time taken to the first potential drop and the number of potential drops are important for the efficiency of virus transmission in case

Table 2 Probing behavior of uninfected and Lso-infected potato psyllids on well-watered and drought-stressed tomato plants during a 12-h recording: mean (± SEM) duration of time (h, min) spent in a particular behavior and total number of probes

	Well-watered		Drought-stressed	
Variable	Uninfected (n = 19)	Infected (n = 18)	Uninfected (n = 22)	Infected (n = 27)
Probing behavior				
Time to first probe (min)	4.7 ± 3.7	0.5 ± 0.4	1.3 ± 0.8	0.3 ± 0.3
No. probes	10.4 ± 1.2	14.0 ± 1.9	17.1 ± 3.3	12.7 ± 1.7
Sieve element phase				
Time from the beginning of the first probe to first D (h)	1.7 ± 0.3	2.6 ± 0.5	3.9 ± 0.8	2.9 ± 0.2
Time from start of EPG to first E1 (h)	3.1 ± 0.7	3.3 ± 0.7	6.0 ± 1.0	4.3 ± 0.8
Time from start of EPG to first E2 (h)	6.1 ± 1.0	4.9 ± 0.8	8.0 ± 0.9	6.9 ± 0.9
Time from start of EPG to first sustained E2 (E2 >10 min) (h)	7.5 ± 1.0	6.8 ± 0.9	9.7 ± 0.7	9.7 ± 0.7
Total duration of E1 followed by E2 (min)	14.5 ± 3.9	25.6 ± 5.6	11.2 ± 2.8	6.7 ± 2.2

Results of the statistical tests are provided in the supplemental material (Table S1).

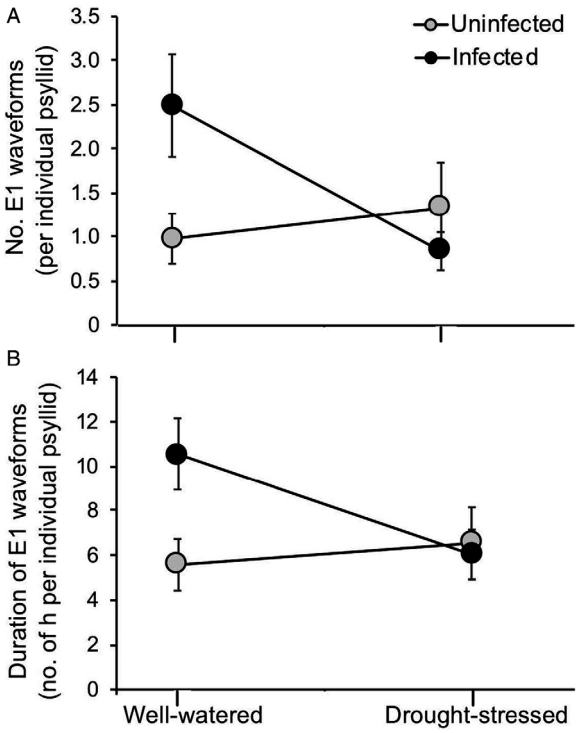

Figure 3 Mean (± SEM) (A) number and (B) duration (h) of phloem salivation (E1) generated by Lso-free and -infected potato psyllids exposed to well-watered and drought-stressed tomato.

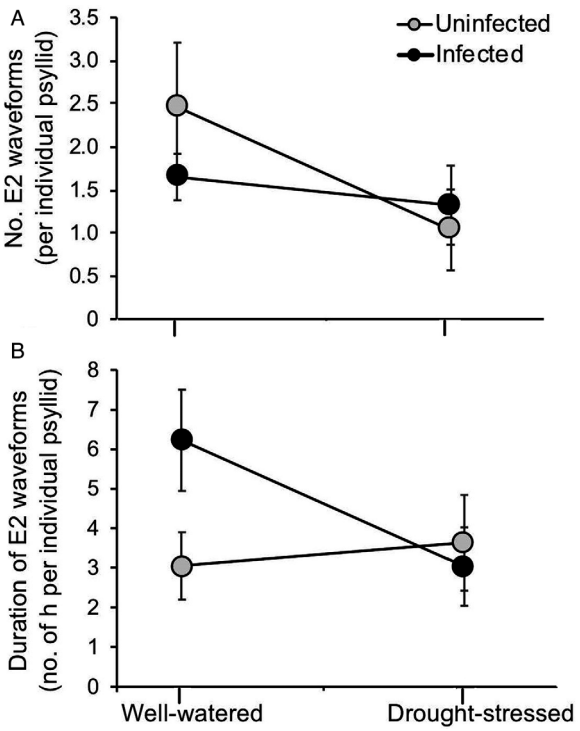

Figure 4 Mean (± SEM) (A) number and (B) duration (h) of phloem ingestion (E2) generated by Lso-free and -infected potato psyllids exposed to well-watered and drought-stressed tomato.

of non-persistently transmitted plant viruses (Martin et al., 1997). On the other hand, the relevance of these variables for transmission of Lso is unclear. Time to first probe was significantly shorter when psyllids were infected with Lso, which was particularly evident in the well-watered plants in our study. A similar outcome was previously reported for bird cherry-oat aphid exposed to several crop and grass species under drought stress (Ponder et al., 2001; Hale et al., 2003). Whereas the probing behavior of these aphids appeared to be unaffected by drought stress in these studies, the phloem salivation (E1) and ingestion (E2) were impacted by the water status of the host plants.

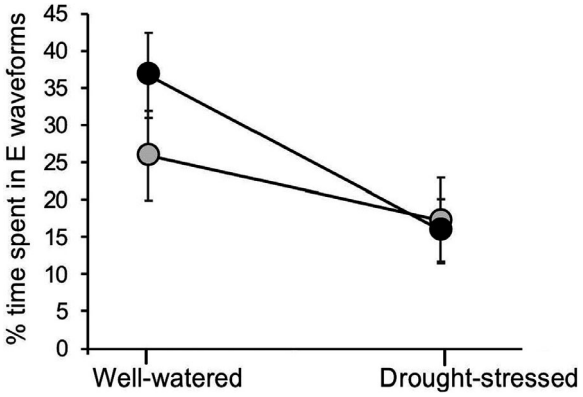

Figure 5 Mean (± SEM) time (%) that the Lso-free and -infected potato psyllids exposed to well-watered or drought-stressed tomato spent in the sieve element phase.

We noted strong and consistent effects of the interaction between drought and Lso infection on the time spent, duration, and number of waveforms associated with phloem salivation and ingestion phases, which are considered key events during which inoculation and acquisition of pathogens, including Lso, occur (Pearson et al., 2014; Sandanayaka et al., 2014; Mustafa et al., 2015). Drought stress exerted the strongest influence on these feeding behaviors, which has been demonstrated previously in other systems (Ponder et al., 2001; Hale et al., 2003; Krugner & Backus, 2014). Notably, we also observed interesting effects of Lso infection on salivation and phloem ingestion. With respect to E1, differences were observed between infected and uninfected potato psyllids only on well-watered plants. There were no differences in the number or duration of waveform E1 between the infected and uninfected psyllids exposed to drought-stressed tomato. A similar trend was observed for E2, where infected psyllids feeding on well-watered plants had higher frequency of E2 behaviors compared to Lso-infected psyllids exposed to drought-stressed plants. Moreover, E1 and E2 phases were reduced under drought stress irrespective of the pathogen infection, suggesting that the impact of water status superseded the effect of Lso infection on salivation and phloem

Table 3 Mean (± SEM) number of waveform events (see Material and methods for their description) in a 12-h recording per individual uninfected and Lso-infected potato psyllid on well-watered (control) or drought-stressed tomato plants

Treatment	NP	C	D	G
Well-watered				
Uninfected	9.9 ± 1.2	12.7 ± 1.2	5.5 ± 1.3	2.2 ± 0.7
Infected	13.4 ± 1.9	15.4 ± 1.8	6.3 ± 1.2	1.9 ± 0.3
Drought-stressed				
Uninfected	16.7 ± 3.3	19.1 ± 3.3	4.6 ± 1	2.0 ± 0.5
Infected	11.8 ± 1.7	16.6 ± 1.8	4.5 ± 0.6	2.1 ± 0.4
Water	$F_{1,81} = 0, P = 0.99$	$F_{1,82} = 0.66, P = 0.81$	$F_{1,78} = 3.62, P = 0.06$	$F_{1,82} = 0, P = 0.95$
Infection	$F_{1,81} = 0.01, P = 0.93$	$F_{1,82} = 0.02, P = 0.89$	$F_{1,78} = 2.17, P = 0.14$	$F_{1,82} = 0.1, P = 0.75$
Water*infection	$F_{1,81} = 0.21, P = 0.64$	$F_{1,82} = 0.05, P = 0.83$	$F_{1,78} = 0, P = 0.96$	$F_{1,82} = 0.30, P = 0.58$

Mixed model ANOVA was used to analyze the effects of treatments on each parameter.

Table 4 Mean (± SEM) duration (h) of waveform events (see Material and methods for their description) during the 12-h recording per Lso-infected and uninfected potato psyllid on well-watered (control) or drought-stressed tomato plants

Treatment	NP	C	D	G
Well-watered				
Uninfected	1.7 ± 0.5	6.1 ± 0.7	0.11 ± 0.03	1.2 ± 0.3
Infected	1.3 ± 0.4	5.5 ± 0.5	0.27 ± 0.11	0.9 ± 0.1
Drought-stressed				
Uninfected	2.4 ± 0.7	6.0 ± 0.5	0.13 ± 0.02	1.3 ± 0.2
Infected	2.1 ± 0.5	6.7 ± 0.5	0.19 ± 0.07	1.1 ± 0.2
Water	$F_{1,82} = 21783.1, P<0.001$	$F_{1,82} = 1930.53, P<0.001$	$F_{1,71} = 83.79, P<0.001$	$F_{1,72} = 1298.71, P<0.001$
Infection	$F_{1,82} = 7874.74, P<0.001$	$F_{1,82} = 124.4, P<0.001$	$F_{1,71} = 4397.16, P<0.001$	$F_{1,72} = 4496.3, P<0.001$
Water*infection	$F_{1,82} = 734.6$, $P<0.001$	$F_{1,82} = 8016.94$, $P<0.001$	$F_{1,71} = 532.49$, $P<0.001$	$F_{1,72} = 18.02$, $P<0.001$

Mixed model ANOVA was used to analyze the effects of treatments on each parameter.

ingestion behavior. Previous research on the impact of drought stress on psyllid performance found that drought-stressed plants had significantly more *B. cockerelli* nymphs and adults than well-watered plants due to higher nymphal survival on these plants (Huot et al., 2013). Whereas the authors did not test the impact of drought stress on performance of Lso-infected and uninfected individuals, they speculated that increased psyllid population would cause adults to move between plants, thereby increasing Liberibacter spread (Huot et al., 2013). Future studies should be aimed at investigating the feeding behavior and performance of vectors as it relates to transmission of a pathogen under varying drought stress levels.

Moreover, Lso infection increased frequency of salivation, which directly benefits pathogen transmission, although these effects were largely observed during well-watered conditions. There is accumulating evidence that pathogens alter vector feeding behavior to enhance transmission and spread. For example, probing behaviors increased in aphids on plants infected with *Cucurbit aphid-borne yellows virus* (Carmo-Sousa et al., 2016) and in two grain aphids infected with *Barley yellow dwarf virus* (Montllor & Gildow, 1986). Similarly, viruliferous whiteflies [*Bemisia tabaci* (Gennadius)] infected with *Tomato yellow leaf curl virus* fed more readily and spent more time salivating into sieve tube elements than uninfected whiteflies (Liu et al., 2013). Likewise, feeding behavior of thrips (*Frankliniella occidentalis* Pergande) increased three-fold after virus acquisition, thus amplifying the probability of virus transmission (Stafford et al., 2011). Overall, our data and previous research demonstrate that drought can have complex and variable effects on the feeding behavior of insect vectors and potentially on the subsequent pathogen spread.

We also report a new waveform H that has not been described previously for *B. cockerelli* and has an unknown biological function. Reports of new and previously undescribed waveforms produced by insects during non-feeding phases but while their stylets are inserted into the plant tissues are not uncommon. For example, Krugner & Backus (2014) reported a group of new waveforms generated by glassy-wing sharpshooter. Additional research in the form of histological analyses to determine salivary sheath termini position in plant tissue is required to determine the possible biological interpretation of the behavior associated with the H waveform.

This is the first report of the effects of drought and infection status on the feeding behavior of *B. cockerelli*. Interactions among drought-stressed host plants, pathogens, and their vectors are exceedingly complex (Szczepaniec & Finke, 2019), and studies such as this one advance our understanding of the consequences of drought and infection to feeding behavior of insect vectors, and hence the spread of pathogens and disease incidence.

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Supporting Information

Additional Supporting Information may be found in the online version of this article: **Table S1** Outcomes of ANOVA tests comparing the effect of drought and Lso infection and their interaction on the additional recorded parameters.