

# Plant water stress intensity mediates aphid host choice and feeding behaviour

JESSICA KANSMAN,<sup>1</sup> VAMSI NALAM,<sup>2</sup> PUNYA NACHAPPA<sup>2</sup> and DEBORAH FINKE<sup>1</sup> <sup>1</sup>Division of Plant Sciences, University of Missouri, Columbia, Missouri, U.S.A. and <sup>2</sup>Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, Colorado, U.S.A.

**Abstract.** 1. The effects of drought-induced changes in plant quality on aphid performance and population growth is well-studied. The response of aphid behaviour to plant water limitation has received less attention. Water limitation may affect host-plant colonization by altering the attractiveness of plants. Additionally, plant water limitation may inhibit feeding site establishment and phloem ingestion.

2. Our goal was to examine bird cherry-oat aphid (*Rhopalosiphum padi* L.) host selection and feeding behaviour under water limitation. We assessed aphid response to well-watered, mildly-stressed, and highly-stressed wheat (*Triticum aestivum* L.) by evaluating (i) host-plant selection through two-choice assays, (ii) feeding behaviour using the electrical penetration graph technique, and (iii) phloem ingestion by quantifying honeydew production.

3. Aphids were less likely to select highly stressed plants than a mildly stressed or well-watered alternative. Aphids did not distinguish between mildly stressed and well-watered plants. Aphid feeding behaviours, including duration of phloem ingestion, were not affected by water availability. However, honeydew production was reduced under both levels of water limitation. These results suggest that the volume of phloem ingested by aphids per unit time declined on stressed plants. The combination of lower colonization and diminished access to food on stressed plants may lead to a reduction in aphid abundance, independent of the direct effects of nutrition on individual aphid performance.

4. This study highlights the potential contribution of herbivore behaviour to documented changes in aphid abundance on stressed plants and underscores the important role of plant water stress intensity in mediating plant-herbivore interactions.

**Key words.** Drought, electrical penetration graph, honeydew, host choice, *Rhopalosiphum padi*, *Triticum aestivum*.

## Introduction

Increasing frequency of drought events driven by global climate change influences the abundance and distribution of organisms across multiple trophic levels (Suttle *et al.*, 2007; Barnett & Facey, 2016; Lu *et al.*, 2016). Insect herbivores experience the effects of water limitation both directly and indirectly through stress-induced changes in the quality of their food plants (Mattson & Haack, 1987). Whether these dietary changes benefit or disadvantage herbivore performance and population

growth varies across species and may depend on factors such as the feeding style of the insect and the timing and duration of water limitation (White, 1969; Larsson, 1989; Huberty & Denno, 2004).

Phloem-feeding insects like aphids are predicted to respond positively to drought due to an increase in the nutritional quality of their food when plants are water-limited (White, 1969). However, studies that investigate aphid performance when feeding on water-stressed plants have shown many possible outcomes, including positive (Oswald & Brewer, 1997; Khan *et al.*, 2010; Mewis *et al.*, 2012; Tariq *et al.*, 2012), negative (Oswald & Brewer, 1997; Hale *et al.*, 2003; Simpson *et al.*, 2012; Tariq *et al.*, 2012; Guo *et al.*, 2016; Nachappa *et al.*, 2016; Pineda *et al.*, 2016), and neutral (Mewis *et al.*, 2012; Banfield-Zanin

Correspondence: Deborah Finke, Division of Plant Sciences, University of Missouri, Columbia, MO 65201, U.S.A. E-mail: finked@missouri.edu

& Leather, 2015). Some of this variation may be attributed to the severity of water limitation and the inability of aphids to access food resources, despite higher nutritional quality, as stress increases and phloem pressure declines (Huberty & Denno, 2004). In addition, the attraction of aphids to particular host plants and their decision to colonise, settle, and feed may be influenced by water limitation. However, drought studies often employ the strategy of measuring aphid performance and population growth when caged on water-limited hosts, overlooking the potentially important contribution of these drought-mediated changes in aphid behaviour to the overall population response.

Water limitation may affect aphid host-plant selection by altering the olfactory, visual, and gustatory cues used by aphids (Döring, 2014). Aphids locate and discriminate among plants using volatile organic compounds (Webster, 2012) and colour (Döring & Chittka, 2007), and both of these traits are known to exhibit water stress-induced variation (Holopainen & Gershenson, 2010; Niinemets, 2010; Taiz *et al.*, 2015). Once a host plant is located and contacted, aphids evaluate plant suitability using chemosensors on the tip of the antennae and by penetrating the plant epidermis with the stylet, ingesting a small amount of cell contents (Powell *et al.*, 2006; Walling, 2008). Water stress influences plant suitability by altering the production of metabolites involved in insect nutrition and plant defense (Showler, 2014), yet evidence of aphid discrimination between stressed and unstressed host plants is lacking (Pineda *et al.*, 2016). However, other insect herbivores, like whiteflies (Inbar *et al.*, 2001) and caterpillars (Inbar *et al.*, 2001; Gutbrodt *et al.*, 2011; Weldegergis *et al.*, 2015; Pineda *et al.*, 2016), make host-selection decisions based on the water status of the plant, so it is reasonable to predict that some aphids do as well.

Once a host plant is selected, drought-induced changes to plant physiology may influence the ability of the aphid to successfully establish a feeding site. Aphids establish feeding sites by secreting a gel-like saliva on the surface of the plant to anchor the beak. They then move their stylet into the plant tissue between cells in search of the phloem sieve elements. During this process, the aphid punctures cells and secrete watery saliva containing effector proteins that are known to interfere with plant defense (Nalam *et al.*, 2019). In the sieve element, these effector proteins block sieve-tube occlusion mechanisms allowing aphids access to a constant stream of phloem contents (Elzinga & Jander, 2013). On poorer quality hosts, aphids show an increase in the number of cell probes and the duration of salivation (Ponder *et al.*, 2001), indicating difficulty in establishing sustained feeding sites. However, studies evaluating phloem feeding behaviour in response to water limitation have found mixed results, with some studies documenting a reduction in the time spent feeding from phloem on water-limited hosts (Guo *et al.*, 2016) and some studies finding no difference in feeding (Ponder *et al.*, 2001; Hale *et al.*, 2003; Nachappa *et al.*, 2016).

Even if aphids are successful in establishing a sustained feeding site, the rate of phloem ingestion may be influenced by water stress. The muscles associated with the stylet are primarily used to direct the stylet through the plant and not for ingestion; therefore, aphids rely on the pressure in the phloem to push phloem contents into the stylet (Auclair, 1963). Water

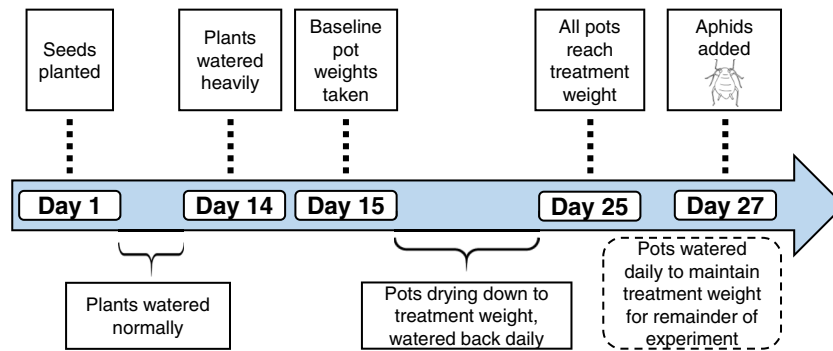
limitation decreases the pressure within the phloem, which is predicted to decrease the rate of phloem intake (Ponder *et al.*, 2001). Furthermore, plants respond to drought-stress conditions by accumulating solutes within the phloem, increasing both sucrose concentrations and phloem viscosity (Lemoine *et al.*, 2013; Sevanto, 2018). Phloem is already a sugar-rich resource, with amino acids comprising only 20% of the sap and a large ratio of nonessential to essential amino acids (Sandström & Moran, 1999; Douglas, 2006). To combat normal concentrations of sucrose and maintain water balance, aphids reduce the osmotic potential of the ingested fluid in their gut by transforming the sugar into products with similar osmotic potential as the aphid hemolymph and excreting most of the sugar as honeydew (Wilkinson *et al.*, 1997). Since aphids ingest far more phloem sap than is assimilated (Douglas, 2006), the production of honeydew is assumed to be directly related to the volume of phloem ingested (Issacs *et al.*, 1998; Hale *et al.*, 2003). Thus, changes in honeydew production could indicate declining feeding efficiency as a result of reduced ingestion rate, osmotic regulation, or a combination of the two (Nalam *et al.*, 2020). Some studies have shown that honeydew production by phloem feeding insects declines when feeding on water-limited plants (Issacs *et al.*, 1998; Hale *et al.*, 2003), but the field is lacking in studies and consensus (Baqui & Kershaw, 1993; Tan *et al.*, 2017).

The goal of this study was to identify the consequences of water limitation for aphid host-plant selection, feeding site establishment, and phloem ingestion. We assessed the behavioural responses of bird cherry-oat aphids (*Rhopalosiphum padi* L.) to persistently water-stressed wheat plants (*Triticum aestivum* L.) in a series of experiments that included choice assays, monitoring feeding behaviour using the electrical penetration graph (EPG) technique, and quantification of honeydew production. This study is novel in that we evaluated aphid behavioural response using two levels of water limitation. Little work has been done to evaluate insect behavioural response to mild reductions in water availability, and it is unclear whether responses will be affected in a linear manner as stress increases (Larsson, 1989). We hypothesised that aphids would preferentially colonise mildly stressed plants due to greater nutrient availability, but avoid highly stressed plants where the lack of sufficient phloem pressure limits access to food. Likewise, we predicted that aphids would have more difficulty establishing and maintaining feeding sites as plant stress increased, resulting in a decrease in phloem ingestion duration and honeydew production. Experimental investigation of the relationship between water limitation and aphid behaviour will contribute to a more complete understanding of the mechanisms underlying aphid population response to drought.

## Materials and methods

### Aphid colony

Bird cherry-oat aphids were reared in cages in the Ashland Road Greenhouse Facility (photoperiod of 16:8, 26–38 °C; University of Missouri, Columbia, Missouri). The colony was established in 2016 from a long-term lab colony held at Kansas State University since 2013 (originally obtained from



**Fig. 1.** Timeline of process to achieve plant water treatments. Plants were grown under well-watered conditions for 14 days. They were then watered to saturation and allowed to drain overnight (12 h). The weight of each pot after the excess water had drained was considered 'baseline'. Water was withheld from pots until pots dried down to a target percent of their weight at baseline: 100% for well-watered, 75% for mild-stress and 50% for high-stress. When the target weight was reached, the pots were watered once daily to maintain this weight. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

Riley County, Kansas). The aphids were maintained on winter wheat variety Coker 9553 (AgriPro) using potting mix (Miracle-Gro Potting Mix) contained within mesh-nylon cages (model BugDorm-2120, dimensions W60 × D60 × H60 cm, mesh size 96 × 261680 μm aperture, MegaView Science Co. Ltd, Taichung, Taiwan). The colony was maintained at low population density by removing plants and adding new ~2-week-old wheat plants several times per week.

#### Drought methods

Three experiments were conducted to evaluate aphid behavioural responses to plants experiencing a gradient of water availability. For the 1st and 3rd experiments, wheat seeds were planted within 19.05 × 18.09 × 15.88 cm pots (3.78 L, Hummert International, Earth City, Missouri) containing 400 g of air-dried growing medium (PRO-MIX BX General Purpose non-mycorrhizae, Riviere-du-Loup, Canada). For the 2nd experiment, 15.24 × 11.43 × 11.43 cm pots (1.6 L) with 200 g of dried growing media were used due to space constraints. Plants were grown in the greenhouse under well-watered conditions for 14 days and fertilised once (All-Purpose Plant Food, Scott's Miracle Gro, Marysville, Ohio) before water treatments were applied (Fig. 1). For all experiments, 14 days after germination (Feekes stage 1.0–2.0; Miller, 1992) the soil was watered to saturation and allowed to drain overnight (12 h). The weight of each pot after the excess water had drained was considered 'baseline' and served as the reference weight for creating water treatments. Pots in the well-watered treatment were weighed and watered daily to maintain the baseline weight (100%). Water was withheld from pots for approximately 7–10 days in the stress treatments until pots dried down to a percent of their weight at baseline: 75% for mild-stress and 50% for high-stress. When the target weight was reached, the pots were watered once daily to maintain this weight. Aphids were not introduced to the plants for a minimum of 48 h after target weights were reached. These water treatments were selected to create a distinct range of water potentials, where photosynthesis may be impacted but plant mortality did not occur during experiments (Gupta *et al.*, 1989). Plant water status was assessed at the end

of each experiment by destructively measuring whole-plant water potential using a pressure chamber (Scholander pressure bomb Model 670, PMS Instrument Company, Albany, Oregon). Water potential was then compared across the 3 water stress treatments using a one-way analysis of variance with a Tukey HSD correction (ANOVA; PROC MIXED, SAS 9.4, SAS Institute, Cary, North Carolina).

*Experiment 1: Aphid host-plant choice.* To assess the effect of water limitation on aphid host-plant selection, we conducted a series of two-choice assays including every combination of water treatment (well-watered vs. mild-stress, well-watered vs. high-stress, mild-stress vs. high-stress). The study took place in the greenhouse [photoperiod of 16:8 (L:D) h, 23–38 °C] using whole plants (Feekes stage 2.0) within nylon and mesh cages (model BugDorm-2120, dimensions W60 × D60 × H60 cm, mesh size 96 × 261680 μm aperture, MegaView Science Co.). Treatment plants were randomly assigned to the left or right side of each cage and an 11.5 × 5 cm cardboard bridge was placed between the pots, configured so the cardboard touched the base of both wheat stems to allow aphids unobstructed access to the plants. Ten adult, apterous aphids were randomly chosen from the colony, placed in the centre of this platform, and allowed 24 h to select a host plant. The number of aphids present on each plant and those that perished during the study were recorded. We conducted at least 21 replications of each treatment combination (well-watered vs. high-stress 22, well-watered vs. mild-stress 21, mild-stress vs. high-stress 23). The experiment was run twice, with 20 cages at Time 1 and 46 cages at Time 2. Since the data did not meet the assumptions required for parametric procedures, a nonparametric test was used. The effects of water treatment pairing and the experimental run (Time 1 or Time 2) on aphid host-plant choice were evaluated using a rank-based Wilcoxon Mann–Whitney test with a Bonferroni correction (PROC NPAR1WAY, SAS 9.4).

*Experiment 2: Aphid feeding behavior.* The feeding behaviour of aphids on well-watered, mildly-stressed, and highly-stress plants was quantified using DC-EPG system

(GIGA 8, EPG Systems, Wageningen, The Netherlands; Tjallingii & Esch, 1993) at Purdue University Fort Wayne (Fort Wayne, Indiana). Adult, apterous aphids randomly chosen from colonies were used for the observations and starved for 1 h before wiring. Aphids were connected to the probe using 18  $\mu\text{m}$  gold wire and silver glue (colloidal silver and paper glue, <https://www.epgsystems.eu/downloads-installfiles-manuals/file/24-add-ons-and-hints>). The 8 copper electrodes were randomly assigned to the plants (Feekes stage 2.0) and placed in the soil at the base of the plant to complete the circuit through the GIGA 8 system. The assays were conducted in a lab setting (23–38 °C, 60–70% humidity), and the plants were enclosed in a copper mesh Faraday cage (60.96  $\times$  60.96  $\times$  91.44 cm). Aphid probing and feeding behaviours were recorded for 8 h using the Stylet+ software (EPG Systems). Recordings were only included in the final analysis if a minimum of 3 h of activity was captured. Additionally, recordings were not included if combined xylem feeding, derailed stylet mechanics, and non-probing duration was >70% of the total reading to avoid increasing the variability of other predictor behaviours (Nalam *et al.*, 2018). We also excluded observations where the aphid became disconnected from the probe, died, or there was an error in the probe connection to the GIGA system. Resulting waveforms from the remaining recordings (well-watered = 14, mild-stress = 11, high-stress = 15) were characterised using Stylet+ A (EPG Systems), and the labels were converted into either time spent in each feeding phase or number of incidences of an action using an automatic parameter calculating macro (Sarría *et al.*, 2009). Since the data did not meet the assumptions required for parametric procedures, differences in the duration of phloem salivation (E1), phloem ingestion (E2), and xylem feeding (G), the number of cell probes (pd), and the time to first sustained (>10 min) phloem ingestion across the three water treatments were assessed using Kruskal-Wallis tests (PROC NPAR1WAY, SAS 9.4).

*Experiment 3: Aphid honeydew production.* Honeydew production by individual aphids was evaluated on well-watered, mildly stressed, and highly stressed plants in the greenhouse [photoperiod of 16:8 (L:D) h, 23–38 °C]. A modified clip-cage arena design was used to restrict aphid movement while providing aphids with leaf material that was still connected to the plant. Arenas were created by cutting a 1.25 cm diameter hole in the side of a 11.43  $\times$  8.89  $\times$  5.08 cm food-storage container (GLAD Designer Series, Proctor & Gamble, Cincinnati, Ohio). A single wheat leaf (Feekes stage 2.0) was inserted into the hole and secured in place with a cotton ball. One adult, apterous aphid randomly chosen from the colony was placed on the underside of the leaf. Wax paper was placed at the bottom of the arena to catch the honeydew falling from the aphid. The centre of the container lid was cut out and replaced with very fine fabric mesh (680  $\mu\text{m}$  aperture) to allow ventilation.

Each of the 3 drought treatments was replicated 16 times across two time periods (time A 10.30 hours–10.30 hours, 7 reps; time B 12.00 hours–12.00 hours, 9 reps) in a randomised complete block design. After 24 h, we counted the number of honeydew drops on the wax paper. Observations were excluded

if the aphid escaped or moved to the top side of the leaf. The effect of the watering treatment on the number of honeydew droplets produced was assessed using one-way ANOVA with blocking for time included as a random factor and a Tukey HSD correction (PROC MIXED, SAS 9.4).

## Results

### *Experiment 1: Aphid host-plant choice*

Water potential was significantly different across treatments ( $F_{2,88} = 139.06$ ,  $P < 0.001$ ; Fig. 2a). Aphid host-plant selection was affected by the intensity of the water-stress treatment. When offered a choice between the two, aphids were equally likely to select well-watered and mildly stressed plants ( $Z = 1.08$ ,  $P = 0.28$ , Fig. 2b). However, aphids selected well-watered and mildly stressed plants over highly stressed plants (well-watered vs. high-stress,  $Z = 3.38$ ,  $P < 0.001$ , Fig. 2c; mild-stress vs. high-stress,  $Z = 2.51$ ,  $P = 0.012$ , Fig. 2d). The effect of the experimental run was not significant, indicating the aphid response was consistent for Time 1 and Time 2.

### *Experiment 2: Aphid feeding behavior*

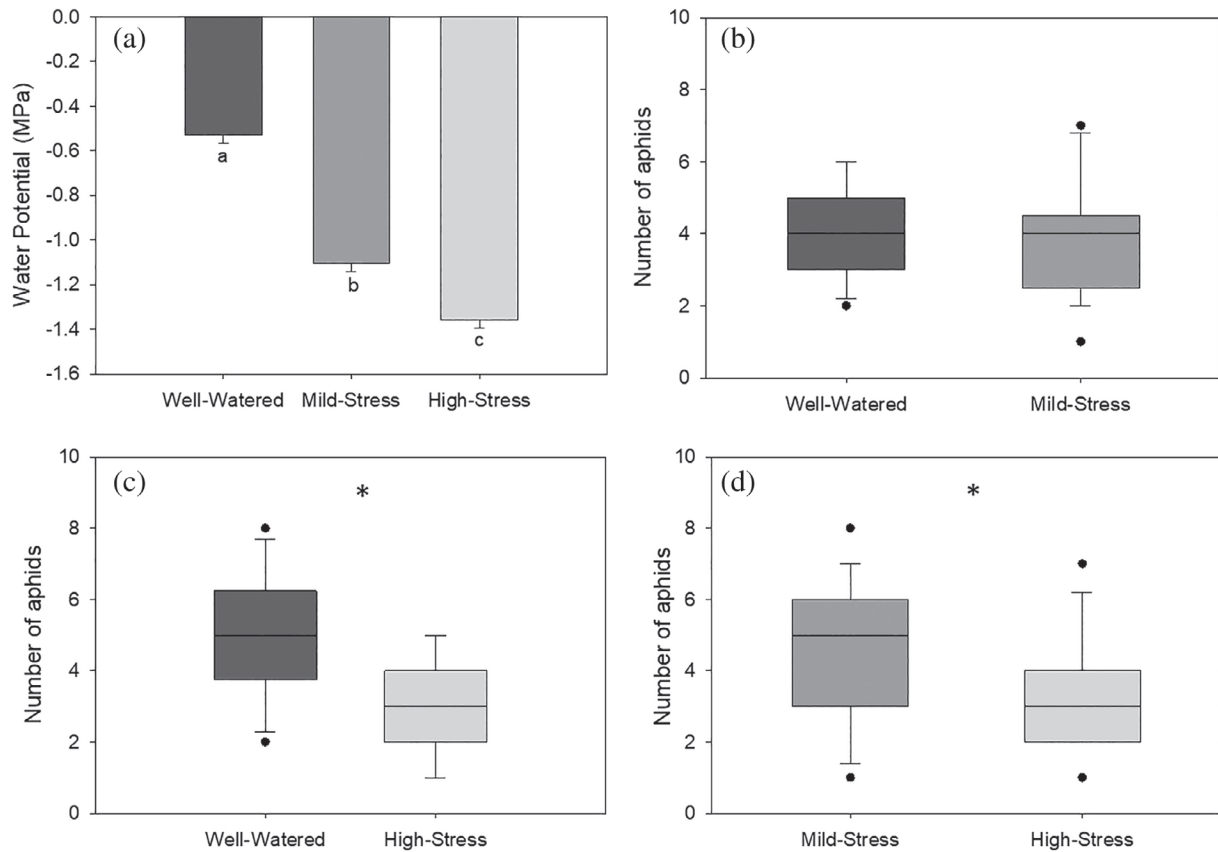
Aphid feeding behaviours were similar across the three watering treatments (Table 1). No differences were detected in the time spent salivating and ingesting phloem sap, time spent feeding in the xylem, or the total number of probes.

### *Experiment 3: Aphid honeydew production*

Water potential was significantly different across treatments ( $F_{2,37} = 116.73$ ,  $P < 0.001$ ; Fig. 3a). Honeydew production was affected by the water-stress treatment ( $F_{2,37} = 5.43$ ,  $P = 0.009$ ; Fig. 3b). Aphids excreted fewer honeydew drops when feeding on stressed plants than well-watered plants, and honeydew production was the same when feeding on mildly stressed and highly stressed plants (well-watered vs. mild-stress  $P = 0.054$ , well-watered vs. high-stress  $P = 0.009$ , mild-stress vs. high-stress  $P = 0.8307$ ).

## Discussion

Many studies explore the effects of stress-induced changes in food quality on insect herbivore performance and population growth (Mattson & Haack, 1987; Koricheva *et al.*, 1998). Fewer studies address the behavioural response of herbivores to water-limited plants and its contribution to patterns of herbivore abundance (Harmon & Barton, 2013). Our study provides evidence that bird cherry-oat aphids consume less food when feeding on water-stressed wheat plants and, when offered a choice, avoid colonizing highly stressed plants. The combination of lower colonization and diminished access to food on stressed plants in the field can lead to a reduction in aphid abundance, independent of the direct effects of nutritional quality on aphid performance.



**Fig. 2.** (a) The effect of watering treatment on whole plant water potential for plants used in the aphid host-plant choice experiment. LSmeans  $\pm$  SEM are shown, and means with different letters designate significance at  $P < 0.05$ . (b–d). The number of aphids out of 10 that colonised a particular plant. Aphids were offered one of three sets of paired choices: (b) well-watered vs. mild-stress, (c) well-watered vs. high-stress, and (d) mild-stress vs. high-stress. The boxplot displays the median as a horizontal line, the interquartile range as a box, the maximum and minimum values as bars or ‘whiskers’, and outliers are indicated with dots. Paired boxplots with \* are significantly different based on  $P < 0.05$  with a Bonferroni correction.

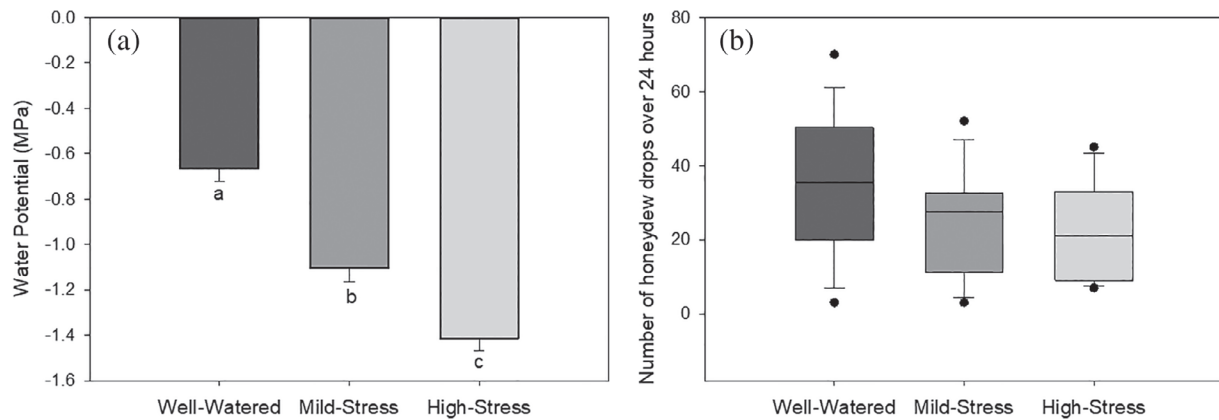
**Table 1.** A summary of the aphid feeding behaviours observed over 8 h using the electrical penetration graph technique.

	Well-Watered (14)	Mild-Stress (11)	High-Stress (15)	H Statistic	<i>P</i>
Total salivation (E1) duration (min)	43.16 (7.65–72.37)*	25.69 (1.91–60.15)	28.87 (9.54–40.63)	0.981	0.613
Total ingestion (E2) duration (min)	234.43 (221.33–271.24)	217.85 (121.27–396.11)	129.86 (101.50–330.92)	0.82	0.664
Total xylem feeding (G) duration (min)	28.40 (0–59.08)	16.43 (0–51.43)	26.83 (0–79.24)	0.352	0.839
Time to first sustained ingestion (sE2) (min)	134.23 (69.35–174.91)	85.76 (60.65–149.55)	168.10 (76.61–268.64)	1.784	0.41
Total number of cell probes (pd)	3.5 (1–5)	7 (5–10)	4 (1–7)	5.756	0.056

\*Due to the use of a non-parametric analysis, the median followed by the interquartile range is presented. The numbers in parentheses indicate sample size for the corresponding treatment.

We found that aphid feeding rate was reduced on water-stressed plants. Aphids were equally successful at establishing feeding sites on stressed and well-watered plants, and the duration of phloem feeding was the same across treatments. However, honeydew production by aphids was lower on stressed plants, indicating a decline in the volume of phloem ingested per unit time. This was true for both mildly and highly stressed plants and suggests a potential constraint on performance on water-limited plants due to reduced resource acquisition. Our results are consistent with previous studies of bird cherry-oat aphids on other grass hosts that found that

aphids fed for the same duration (Ponder *et al.*, 2001; Hale *et al.*, 2003), but less phloem was ingested on water-stressed plants (Hale *et al.*, 2003). Aphid performance declined on water-stressed plants, despite an increase in plant nutritional quality, because aphids ate less food overall (Hale *et al.*, 2003). The changes in aphid feeding behaviour that we document may contribute to altered aphid performance on water-stressed plants as well. Many empirical studies have demonstrated reduced herbivore performance under continuous water limitation (Issacs *et al.*, 1998; Inbar *et al.*, 2001; Hale *et al.*, 2003; Simpson *et al.*, 2012; Tariq *et al.*, 2012; Guo *et al.*, 2016;



**Fig. 3.** (a) The effect of watering treatment on whole plant water potential for plants used in the aphid honeydew production experiment. LS means  $\pm$  SEM are shown, and means with different letters designate significance at  $P < 0.05$ . (b) The number of honeydew droplets produced by aphids over 24 h compared across three levels of plant water availability. The boxplot displays the median as a horizontal line, the interquartile range as a box, the maximum and minimum values as bars or 'whiskers', and outliers are indicated with dots.

Nachappa *et al.*, 2016; Pineda *et al.*, 2016), and the mechanisms commonly attributed are reduced turgor pressure and increased viscosity negatively affecting phloem access, ingestion rate, or osmoregulation.

Counter to previous predictions, we found that honeydew production declined under mild water limitation. The pulsed stress hypothesis posits that phloem-feeding herbivores benefit from feeding on plants experiencing mild or intermittent water limitation due to an increase in the nutritional quality of the food ingested; however, high or continuous water limitation reduces herbivore performance because access to food is inhibited due to low turgor pressure (Huberty & Denno, 2004). Although the exact turgor pressure threshold for aphids is unclear, our results suggest that even subtle changes in water availability influence feeding efficiency and could ultimately impact aphid performance, not by changing food quality, but by limiting the quantity of food ingested.

Aphid host-selection behaviour was altered only in response to high water stress, despite evidence of reduced feeding efficiency under all levels of water limitation. As predicted, aphids were less likely to colonise a highly stressed plant than either a mildly stressed or well-watered alternative. However, aphids colonised mildly stressed and well-watered plants at similar rates. Our finding of consistent selection of mildly-stressed plants by aphids despite a reduction in feeding efficiency is similar to the result of Pineda *et al.* (2016) that green peach aphids (*Myzus persicae* (Sulzer)) did not distinguish among *Arabidopsis thaliana* plants based on water availability despite a performance decline on highly-stressed plants. Taken together, these results suggest that plant traits associated with aphid preference are not directly related to those involved with performance. This is reasonable to conclude since the production of volatile organic compounds used as cues for host location and of metabolites involved in insect nutrition and plant defense respond to the intensity and the duration of water stress, but not necessarily in a dose-dependent fashion (Niinemets, 2010; Tariq *et al.*, 2012; Pineda *et al.*, 2016).

Consequently, abiotic stress may affect the reliability of plant cues herbivores use to determine host quality.

Changing host-plant condition in response to abiotic stress events can alter plant-herbivore interactions (Inbar *et al.*, 2001; Hale *et al.*, 2003; Huberty & Denno, 2004; Pineda *et al.*, 2016). However, our ability to predict the outcomes of these interactions is limited because many studies only test the presence or absence of drought and ignore variation in timing and intensity (Larsson, 1989). Our study found that herbivore host-plant choice and feeding rate, indicators of herbivore preference and performance, respond differently to stress, with feeding rate being relatively more sensitive to small changes in water availability. This result was only revealed because water availability was manipulated to create a gradient of stress conditions and would have been missed using traditional experimental designs. Climate change scenarios predict that drought events will increase in both frequency and variability (Prein *et al.*, 2016; Pendergrass *et al.*, 2017), with major implications for insect populations. However, the mechanisms critical for driving population level insect responses are still under investigation. By advancing our understanding of herbivore behavioural responses to plants across a range of water-stress intensity, we improve our ability to predict how herbivore populations will respond to fluctuating weather conditions.

### Acknowledgements

We thank Bruce Hibbard, Felix Fritschi, and Abe Koo for contributing to the development of the research and providing comments on the manuscript. Additionally, we thank Travis Issacs, Sarah Moh, Harper Smith, Kristin Tosie, Mason Ward, Nicole Pruess, Nick Rector, and Jared Brabant for their assistance with equipment training and data collection. This project was supported by the University of Missouri Research Board and USDA NIFA MO-HAPS0006. Additionally, this work was supported by AFRI EWD (2019-67011-29729) from the USDA National

Institute of Food and Agriculture. The authors have no conflicts of interest to declare.

### Author contribution

The project was designed by JK and DF. Training for data collection and analysis of the EPG component of the project was facilitated by VN and PN. JK was responsible for all other analyses, data collection, and paper writing with critical input from DF. VN and PN also provided valuable comments on the manuscript.

### Data availability statement

The data that supports the findings of this study are openly available in MOpenSpace data repository at <https://doi.org/10.32469/10355/74881>.

### References

- Auclair, J.L. (1963) Aphid feeding and nutrition. *Annual Review of Entomology*, **8**, 439–490.
- Banfield-Zanin, J.A. & Leather, S.R. (2015) Drought intensity and frequency have contrasting effects on development time and survival of the green spruce aphid. *Agricultural and Forest Entomology*, **17**, 309–316.
- Baqui, M.A. & Kershaw, W.J.S. (1993) Effect of plant water stress on honeydew production, weight gain and oviposition of brown planthopper *Nilaparvata lugens* on rice cultivars. *Entomologia Experimentalis et Applicata*, **67**, 25–30.
- Barnett, K.L. & Facey, S.L. (2016) Grasslands, invertebrates, and precipitation: a review of the effects of climate change. *Frontiers in Plant Science*, **7**, 1196.
- Döring, T.F. (2014) How aphids find their host plants, and how they don't. *Annals of Applied Biology*, **165**, 3–26.
- Döring, T.F. & Chittka, L. (2007) Visual ecology of aphids—a critical review on the role of colours in host finding. *Arthropod-Plant Interactions*, **1**, 3–16.
- Douglas, A.E. (2006) Phloem-sap feeding by animals: problems and solutions. *Journal of Experimental Botany*, **57**, 747–754.
- Elzinga, D.A. & Jander, G. (2013) The role of protein effectors in plant–aphid interactions. *Current Opinion in Plant Biology*, **16**, 451–456.
- Guo, H., Sun, Y., Peng, X., Wang, Q., Harris, M. & Ge, F. (2016) Up-regulation of abscisic acid signaling pathway facilitates aphid xylem absorption and osmoregulation under drought stress. *Journal of Experimental Botany*, **67**, 681–693.
- Gupta, A.S., Berkowitz, G.A. & Pier, P.A. (1989) Maintenance of photosynthesis at low leaf water potential in wheat. *Plant Physiology*, **89**, 1358–1365.
- Gutbrodt, B., Mody, K. & Dorn, S. (2011) Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos*, **120**, 1732–1740.
- Hale, B.K., Bale, J.S., Pritchard, J., Masters, G.J. & Brown, V.K. (2003) Effects of host plant drought stress on the performance of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.): a mechanistic analysis. *Ecological Entomology*, **28**, 666–677.
- Harmon, J.P. & Barton, B.T. (2013) On their best behavior: how animal behavior can help determine the combined effects of species interactions and climate change. *Annals of the New York Academy of Sciences*, **1297**, 139–147.
- Holopainen, J.K. & Gershenzon, J. (2010) Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science*, **15**, 176–184.
- Huberty, A.F. & Denno, R.F. (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, **85**, 1383–1398.
- Inbar, M., Doostdar, H. & Mayer, R.T. (2001) Suitability of stressed and vigorous plants to various insect herbivores. *Oikos*, **94**, 228–235.
- Issacs, R., Byrne, D.N. & Hendrix, D.L. (1998) Feeding rates and carbohydrate metabolism by *Bemisia tabaci* (Homoptera: Aleyrodidae) on different quality phloem saps. *Physiological Entomology*, **23**, 241–248.
- Khan, M.A.M., Ulrichs, C. & Mewis, I. (2010) Influence of water stress on the glucosinolate profile of Brassica oleracea var. italica and the performance of *Brevicoryne brassicae* and *Myzus persicae*. *Entomologia Experimentalis et Applicata*, **137**, 229–236.
- Koricheva, J., Larsson, S., Haukioja, E. & Keinänen, M. (1998) Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos*, **83**, 212–226.
- Larsson, S. (1989) Stressful times for the plant stress: insect performance hypothesis. *Oikos*, **56**, 277–283.
- Lemoine, R., La Camera, S., Atanassova, R., Deedaldeechamp, F., Allario, T., Pourtau, N. *et al.* (2013) Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in Plant Science*, **4**, 21.
- Lu, X., Gray, C., Brown, L.E., Ledger, M.E., Milner, A.M., Mondragón, R.J. *et al.* (2016) Drought rewires the cores of food webs. *Nature Climate Change*, **6**, 875–878.
- Mattson, W.J. & Haack, R.A. (1987) The role of drought in outbreaks of plant-eating insects. *Bioscience*, **37**, 110–118.
- Mewis, I., Khan, M.A.M., Glawischign, E., Schreiner, M., Ulrichs, C. (2012) Water Stress and Aphid Feeding Differentially Influence Metabolite Composition in Arabidopsis thaliana (L.). *PLoS ONE*, **7**, e48661.
- Miller, T.D. (1992) Growth stages of wheat. *Better crops with plant food*, **76**, 12.
- Nachappa, P., Culkin, C.T., Saya, P.M., Han, J. & Nalam, V.J. (2016) Water stress modulates soybean aphid performance, feeding behavior, and virus transmission in soybean. *Frontiers in Plant Science*, **7**, 552.
- Nalam V., Isaacs T., Moh S., Kansman J., Finke D., Albrecht T., & Nachappa P. (2020) Diurnal feeding as a potential mechanism of osmoregulation in aphids. *Insect Science*, <http://dx.doi.org/10.1111/1744-7917.12787>.
- Nalam, V., Louis, J., Patel, M. & Shah, J. (2018) Arabidopsis-green peach aphid interaction: rearing the insect, no-choice and fecundity assays, and electrical penetration graph technique to study insect feeding behavior. *Bio-Protocol*, **8**, e2950.
- Nalam, V., Louis, J. & Shah, J. (2019) Plant defense against aphids, the pest extraordinaire. *Plant Science*, **279**, 96–107.
- Niinemets, U. (2010) Mild versus severe stress and BVOCs: thresholds, priming and consequences. *Trends in Plant Science*, **15**, 145–153.
- Oswald, C.J. & Brewer, M.J. (1997) Aphid–barley interactions mediated by water stress and barley resistance to Russian wheat aphid (Homoptera: Aphididae). *Environmental Entomology*, **26**, 591–602.
- Pendergrass, A.G., Knutti, R., Lehner, F., Deser, C. & Sanderson, B.M. (2017) Precipitation variability increases in a warmer climate. *Scientific Reports*, **7**, 17966–17966.
- Pineda, A., Pangesti, N., Soler, R., Dam, N.M.v., Loon, J.J.A.v. & Dicke, M. (2016) Negative impact of drought stress on a generalist leaf chewer and a phloem feeder is associated with, but not explained by an increase in herbivore-induced indole glucosinolates. *Environmental and Experimental Botany*, **123**, 88–97.
- Ponder, K.L., Pritchard, J., Harrington, R. & Bale, J.S. (2001) Feeding behaviour of the aphid *Rhopalosiphum padi* (Hemiptera: Aphididae)

- on nitrogen and water-stressed barley (*Hordeum vulgare*) seedlings. *Bulletin of Entomological Research*, **91**, 125–130.
- Powell, G., Tosh, C.R. & Hardie, J. (2006) Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, **51**, 309–330.
- Prein, A.F., Rasmussen, R.M., Ikeda, K., Liu, C., Clark, M.P. & Holland, G.J. (2016) The future intensification of hourly precipitation extremes. *Nature Climate Change*, **7**, 48.
- Sandström, J. & Moran, N. (1999) How nutritionally imbalanced is phloem sap for aphids? *Proceedings of the 10th International Symposium on Insect-Plant Relationships* (ed. by b. S. J. Simpson, A. J. Mordue and J. Hardie), pp. 203–210. Springer, Dordrecht, The Netherlands.
- Sarria, E., Cid, M., Garzo, E. & Fereres, A. (2009) Excel workbook for automatic parameter calculation of EPG data. *Computers and Electronics in Agriculture*, **67**, 35–42.
- Sevanto, S. (2018) Drought impacts on phloem transport. *Current Opinion in Plant Biology*, **43**, 76–81.
- Showler, A.T. (2014) Plant-arthropod interactions affected by water deficit stress through association with changes in plant free amino acid accumulations. *Molecular Approaches in Plant Abiotic Stress* (ed. by b. R. K. Gaur and P. Sharma), pp. 299–352. CRC Press, Boca Raton, Florida.
- Simpson, K.L.S., Jackson, G.E. & Grace, J. (2012) The response of aphids to plant water stress – the case of *Myzus persicae* and *Brassica oleracea* var. *capitata*. *Entomologia Experimentalis et Applicata*, **142**, 191–202.
- Suttle, K.B., Thomsen, M.A. & Power, M.E. (2007) Species interactions reverse grassland responses to changing climate. *Science*, **315**, 640–642.
- Taiz, L., Zeiger, E., Moller, I.M. & Murphy, A.S. (2015) *Plant Physiology and Development*, 6th edn. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
- Tan, Y., Zhu, M., Xu, W., Zhou, W., Lu, D., Shang, H. *et al.* (2017) Influence of water-stressed rice on feeding behavior of brown planthopper, *Nilaparvata lugens* (Stål). *Journal of Asia-Pacific Entomology*, **20**, 665–670.
- Tariq, M., Wright, D.J., Rossiter, J.T. & Staley, J.T. (2012) Aphids in a changing world: testing the plant stress, plant vigour and pulsed stress hypotheses. *Agricultural and Forest Entomology*, **14**, 177–185.
- Tjallingii, W.F. & Esch, T.H. (1993) Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiological Entomology*, **18**, 317–328.
- Walling, L.L. (2008) Avoiding effective defenses: strategies employed by phloem-feeding insects. *Plant Physiology*, **146**, 859–866.
- Webster, B.E.N. (2012) The role of olfaction in aphid host location. *Physiological Entomology*, **37**, 10–18.
- Weldegergis, B.T., Zhu, F., Poelman, E.H. & Dicke, M. (2015) Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. *Oecologia*, **177**, 701–713.
- White, T.C.R. (1969) An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology*, **50**, 905–909.
- Wilkinson, T., Ashford, D., Pritchard, J. & Douglas, A. (1997) Honeydew sugars and osmoregulation in the pea aphid *Acyrtosiphon pisum*. *Journal of Experimental Biology*, **200**, 2137.

Accepted 4 July 2020

First published online 19 August 2020

Associate Editor: Alison Karley