

Effects of Atmospheric Pressure Trends on Calling, Mate-Seeking, and Phototaxis of *Diaphorina citri* (Hemiptera: Liviidae)

N. Y. ZAGVAZDINA,¹ T. M. PARIS,² B. J. UDELL,¹ M. STANISLAUSKAS,¹ S. McNEILL,³
S. A. ALLAN,¹ AND R. W. MANKIN^{1,4}

Ann. Entomol. Soc. Am. 108(5): 762–770 (2015); DOI: 10.1093/aesa/sav069

ABSTRACT Insects and other animals sometimes modify behavior in response to changes in atmospheric pressure, an environmental cue that can provide warning of potentially injurious windy and rainy weather. To determine if *Diaphorina citri* (Hemiptera: Liviidae) calling, mate-seeking, and phototaxis behaviors were affected by atmospheric pressure, we conducted analyses to correlate responsiveness with pressure trends over periods up to 48 h before laboratory bioassays. Mean responsiveness increased or decreased depending on the magnitudes and directions of pressure changes measured over different periods up to 24 h before bioassays, and changed differently in calling and mate-seeking bioassays than in phototaxis bioassays. For example, mean responsiveness decreased in mating behavior bioassays but increased in phototaxis bioassays when atmospheric pressure changed more than one standard deviation over a 24-h period. Such a result is consistent with a hypothesis that there may be survival benefits to focusing energy on dispersal or migration rather than mating after occurrences of sustained, unusual pressure changes. A finding that mean phototactic responses increased when pressure decreased over 9–24-h periods before bioassays is potentially of practical interest. More knowledge about the effects of atmospheric pressure and other environmental variables on behavior can lead to improved models of psyllid movement or other pest management tools as well as to improved timing of application of pest management tools.

KEY WORDS Acoustics, vibration, psyllid, citrus, weather

Weather conditions involving heavy winds and precipitation or large changes in temperature can cause insect mortality, reduce foraging success, or impair ability to mate (Cornell and Hawkins 1995). Decreasing pressure often is followed by stormy, unsettled weather 12–24 h afterwards (Williams 1997, Burch 2013). Insects are known to cue on multiple environmental variables that are correlated with impending weather changes, including atmospheric pressure (Cowley 1987, Southwick and Moritz 1987, Fredeen and Mason 1991). The ability of insects and other animals to respond behaviorally to approaching storms has interested researchers for centuries (Noverre Press 1834). Numerous studies have been published that describe correlations between atmospheric pressure or pressure trends and variability in behaviors of insects or other animals in the field or laboratory, including feeding behavior (Leskey and Propoky 2003), flight initiation (Fournier et al. 2005), and olfactory response (Steinberg et al. 1992). Some studies

have focused on behavior in relation to immediate pressure magnitudes or short-term pressure trends (Kolyer and Palmer 1968, Paige 1995, Witter et al. 2012), and others have considered effects of longer-term pressure trends (Pellegrino et al. 2013). Owing to the variation in study organisms, target behaviors, and approaches to analysis, the effects of atmospheric pressure on insect behavior remain only partly characterized, and the understanding of such effects would benefit from further study.

The Asian citrus psyllid (*Diaphorina citri* Kuwayama; Hemiptera: Liviidae) began to receive significant attention from researchers in the United States after it was discovered in southeastern United States in 1998 (Halbert and Manjunath 2004), and the devastating citrus disease it vectors, huanglongbing, began spreading through commercial citrus groves in 2005 (Bové 2006, Grafton-Cardwell et al. 2013). Investigators of *D. citri* behavior often avoid conducting laboratory bioassays during periods of unsettled weather after observing apparent correlations between weather trends and instances of either lethargic behavior with reduced responsiveness to stimuli, similar to those reported by Ankney (1984) and Crespo and Castelo (2012), or conversely, instances of elevated irritability and dispersal from the bioassay arena. Light, temperature, and humidity are regulated inside air-conditioned laboratories; consequently, atmospheric pressure is the outdoor

¹US Department of Agriculture (USDA), Agriculture Research Service (ARS), Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), 1700 SW 23rd Dr., Gainesville, FL 32608.

²Entomology and Nematology Department, University of Florida (UF), Gainesville, FL 32611-0620.

³Division of Science and Mathematics, Union College, Lincoln, NE 68506.

⁴Corresponding author, e-mail: Richard.Mankin@ars.usda.gov.

environmental cue most likely to transfer unaltered to the bioassay arena and influence behavior. In a long-term series of laboratory bioassays on *D. citri* courtship behaviors and phototaxis, we had an opportunity to explore in detail the presence or absence of correlations between atmospheric pressure trends and behavior.

During courtship, mate-seeking *D. citri* produce vibrational signals in a duetting pattern of male calls and female replies that assist males searching for females on host plants (Wenninger et al. 2009a). A psyllid caller system has been developed using a microcontroller platform (Arduino Uno, Arduino Inc., Italy) that listens for male calls with a microphone attached to the plant. When a call is detected, the microcontroller signals a piezoelectric buzzer to produce a synthetic mimic of the female duetting reply that elicits additional calls and searching activity by the male (Mankin et al. 2013, Rohde et al. 2013). We have assessed the system in numerous bioassays as a possible tool to monitor or trap males searching for females on branches within citrus tree canopies.

In addition to vibrational signals, light and color are known to be important cues for *D. citri* orientation (Wenninger et al. 2009b, Mangan and Chapa 2013, Paris et al. 2015). We have been assessing psyllid phototactic behaviors in a two-choice vertical arena to identify highly attractive light and color cue combinations for optimizing *D. citri* captures in sticky traps.

For this report, we analyzed relationships between atmospheric pressure trends and variations in the percentages of *D. citri* responding in courtship behavior and phototaxis bioassays. An initial hypothesis was that small pressure changes are unlikely to affect behavior strongly because, even in absence of significant meteorological events, pressure fluctuates in a relatively predictable pattern semi-diurnally (Harris 1954). Consequently, it was a goal to assess behavioral changes particularly during periods when atmospheric pressure or pressure trends had deviated from values expected for normal pressure fluctuation. Values outside the normal ranges might furnish cues triggering responses that increased survival or reproductive fitness.

Materials and Methods

Atmospheric Pressure. Pressure data were obtained from a weather station (model WRL-25, Texas Weather Instruments, East Pilot Point, TX) located 1.32 km from the United States Department of Agriculture (USDA), Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), where mate-seeking and phototaxis bioassays were conducted. The pressure at the beginning of each trial; the pressure trend over time lags of 3, 6, 9, 12, 24, 36, and 48 h before the trial; and the mean and standard deviation (SD) of pressure and pressure-trend were compiled in a spreadsheet for each trial. To confirm that pressure changes indoors were comparable with weather station measurements, pressure data logged by the weather station were compared with pressure data simultaneously logged indoors by a handheld logging device (model B1100-1, Gulf Coast Data

Concepts, Waveland MS). Two 72-h data sets logged at 60-s intervals were obtained at different times from the bioassay arena and from the greenhouse where the psyllids were reared. The indoor-outdoor measurements were compared by regression analysis in Excel (Microsoft Office 2013).

Calling and Mate-Seeking Behavior Bioassays. Behavioral observations of 131 individual *D. citri* calling and searching bouts were conducted on 70 d over a 400-d period. The psyllids were obtained from a huanglongbing-free colony maintained in the CMAVE greenhouse, originally obtained from the USDA, Agricultural Research Service, United States Horticultural Research Laboratory, Fort Pierce, FL. Late-instar nymphs were isolated and placed onto individual *Citrus sp.* seedlings, as described by Paris et al. (2013). After eclosion, adults were sexed and unmated males between 4 and 14 d post-eclosion were tested. Bioassays were performed inside a vibration-shielded anechoic chamber (Mankin et al. 1996), between 8:00 a.m. and 6:00 p.m. Psyllids were placed individually on a leaf of a potted, 30-cm-high *Murraya exotica* L. (Sapindales: Rutaceae) plant. Light was supplied by three 60-W flood lamps approximately 1 m above the plant. A psyllid-caller system similar to that described by Mankin et al. (2013) was used to emit recorded or synthetic mimics of female duetting replies. An Arduino microcontroller platform and a small amplifier-circuit board were connected together, forming a single unit (6 by 5.4 by 2 cm). This controlled the sound-producing piezoelectric buzzer (9S3164, Taiyo Yuden, Tokyo, Japan) that was attached directly to the plant using alligator and/or binder clips. The piezo output produced multiple harmonics between 0.2 and 2 kHz, which have previously been shown to elicit *D. citri* responses (Rohde et al. 2013). Signals were triggered manually using software written for the microcontroller (Bug Phone 2.2 ©2013). Trial length and signal control varied, but generally the trials were approximately 30 min in duration and signals were emitted immediately following a male call, or between 1 and 5 min in absence of calling. Psyllids that jumped or flew off the plant before testing began were not included in the experiment.

To reduce background noise, the chamber door was closed after the psyllid was placed on the plant, and visual observations took place on a monitor outside of the anechoic chamber from footage captured by a video camera (model HDR-SR1, Sony Corp., New York, NY), which was focused on the plant. An accelerometer (model 4371, Brüel & Kjær [B&K], Naerum, Denmark) attached to the base of the plant by an alligator clip system (Mankin et al. 2004) was used to continuously monitor vibrations. Vibrational signals were amplified 30 dB with a charge amplifier (model NEXUS 2692, B&K or similar), band-pass filtered between 500 Hz and 10 kHz, recorded at a rate of 44.1 kHz (model HD-P2, TASCAM, TEAC America, USA). The signals were transferred to a computer located outside the anechoic chamber where oscillograms and spectrograms were displayed using Raven 1.4 software (Charif et al. 2008).

Calling activity and searching activity were recorded and categorized into discrete classes. For calling activity, males that were unresponsive or called at a mean rate of less than three calls per minute were scored as 0, whereas those that actively called with a mean rate of three or more calls per minute over the course of the trial were scored as 1. Searching activity was scored as: no movement or a mean distance of less than 9 mm covered per min, 0; a mean distance of 9 mm or more (approximately three or more psyllid body lengths) covered per min, 1.

For each test of correlation between calling or searching activity and pressure/pressure-trend, the trials were subdivided into three categories. Trials that occurred when the pressure/pressure-trend was within 1 SD of the mean value over the period of all bioassays were categorized as occurring under steady conditions. Those that occurred when the pressure/pressure-trend fell 1 SD below the mean were categorized as occurring under falling pressure/pressure-trend conditions, and trials when the pressure/pressure-trend rose 1 SD above the mean were categorized as occurring under rising pressure/pressure-trend conditions. Contingency analysis (Pearson χ^2) was performed for each pressure/pressure-trend correlation using JMP (SAS Institute Inc. 2013) to test the hypothesis that the rate of calling or searching activity was the same for each pressure/pressure-trend condition. A binomial test of equal proportions with a Šidák correction for multiple comparisons (SAS Institute Inc. 2004) was conducted on comparisons that were found to be significant by the χ^2 analysis.

Phototaxis Bioassays. The phototaxis arena consisted of a black polyvinylchloride pipe (30.5 by 17 cm) with a release chamber (33-ml glass tube) at its base. At the top of the arena was a polystyrene petri dish (150 by 15 mm) sprayed with Tanglefoot[®] aerosol (Comtech, Grand Rapids, MI). Two-choice tests were conducted using different combinations of translucent light filters, green (no. 88) or yellow (no. 10, 4350) (Roscolux, Rosco Labs, Sun Valley, CA), placed directly on the petri tray.

Forty trials were conducted, with each replicate containing a mixed-age cohort of 20–40 adults that were dark-adapted for 20 min prior to the trial. *D. citri* were placed in the release vial and allowed 45 min to walk or fly toward the filtered-light targets at the top of the arena. Psyllids trapped on the coated petri dishes were considered responsive. At the end of the trial, responsive and unresponsive individuals were counted and sexed. In addition, the mean percentages responding to light under different pressure/pressure-trend conditions were assessed by analysis of variance. A Tukey's HSD analysis was conducted on the comparisons when the *F*-test was significant.

Results

In this study, changes in *D. citri* bioassay responsiveness were correlated significantly with rising or falling barometric conditions that exceeded the SD of the changes observed in 3–48-h periods preceding the

bioassays. The directions of the changes were different between the mating behavior bioassays and the phototaxis bioassays. We first consider the magnitudes and ecological context of the pressure changes that occurred during the experiments and then describe the effects on mating and phototactic behaviors.

Atmospheric Pressure Variability Preceding Bioassays. The weather station was near enough to the bioassay arenas that its changes in pressure over time matched with simultaneous changes on a logger placed in both the anechoic chamber and greenhouse (Fig. 1C), though initial values differed slightly (Fig. 1A and B). The 72-h pressure changes in Figure 1A and B represent natural semidiurnal atmospheric pressure fluctuation during the Florida summer, and we did not expect this type of variation to affect behavior. Therefore, we subdivided the trials into groups where pressure and pressure change values fell inside or outside the expected variability during the period of bioassays, identifying trials where the pressure and pressure change values exceeded or fell below 1 SD from the mean (Table 1).

Effects of Falling, Steady, and Rising Pressure Trends on Psyllid Bioassay Responses. The mean percentages of male *D. citri* performing calling, searching, or phototaxis under conditions of falling, steady, or rising pressure trends are shown in Figure 2. Contingency analyses (Table 2) revealed significant differences in mean percentages of calling and searching behaviors under falling, steady, and rising pressure trend conditions 24 h before the calling and searching behavior trials, with binomial tests for equal proportions indicating that the mean calling and searching responses were lower for rising pressure trend than for steady conditions. There also were significant differences in mean percentages of calling by males exposed to falling, steady, and rising pressure trends 12 h before trials, with greater responses under falling pressure conditions than under steady and rising pressure conditions. Finally, the mean percentages of males searching when the pressure was falling or rising were significantly greater than the mean percentages when the pressure was steady at the time of trials. There were no significant differences among mean percentages categorized on the basis of pressure trends measured at other time lags before trials.

No evidence was found for sex effects on *D. citri* phototaxis ($r = 0.74$, $P < |r| = 0.6097$, $n = 50$); consequently, the responses of males and females in the phototaxis experiments were pooled. We hypothesized that the effects of atmospheric pressure on responses to both color targets were similar in each bioassay, so the counts of responses to the two lights also were pooled. The mean total response of 1,154 *D. citri* tested in the phototaxis bioassays was $54.5 \pm 1.97\%$ per replicate. The mean responses for psyllids exposed to falling, steady, and rising pressure trends are shown in Figure 2C. Analysis of variance (Table 3) revealed significant differences in mean percentages of *D. citri* phototaxis under falling, steady, and rising conditions 9, 12, and 24 h before trials, but not for trends at other time lags. Tukey's HSD tests indicated that the mean phototaxis

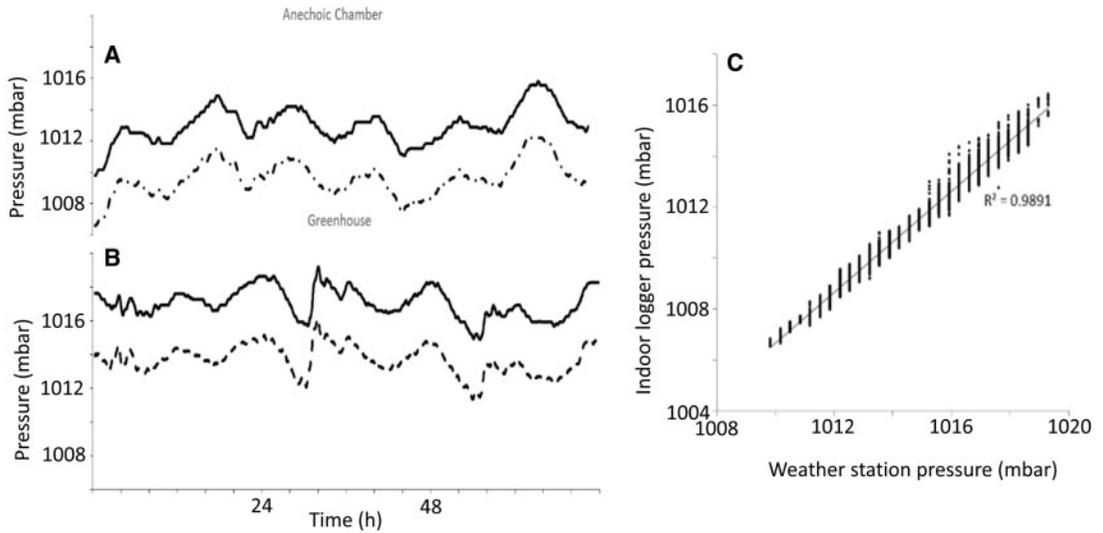


Fig. 1. Pressure values from indoor logger in A) anechoic chamber (dash-dotted line); B) greenhouse (dashed line) compared with weather station output for two 72-h periods (solid line), and C) simple regression showing relationship between pressure values obtained from the weather station and indoor pressure logger, pooling the greenhouse and anechoic chamber readings ($y=0.9955x+1.144$, x =weather station output, y =indoor pressure logger output, residual mean-square error = 0.0561).

Table 1. Means and SD of absolute pressure (mbar) at the time of bioassay trial and changes in pressure (mbar) from 3–48 h prior to trial (time lag)

Time lag (h)	Calling/Searching ($n = 131$) Mean (SD)	Phototaxis ($n = 40$) Mean (SD)
0	1016.58 (4.57)	1013.85 (2.91)
3	-1.0 (1.15)	-0.60 (1.22)
6	-0.59 (1.99)	-0.06 (2.14)
9	0.16 (1.84)	0.17 (2.54)
12	0.13 (1.94)	-0.29 (2.88)
24	-0.27 (3.74)	-1.07 (3.87)
36	0.14 (5.12)	-0.85 (4.23)
48	-0.14 (5.76)	-1.20 (4.60)

response was greater under falling pressure conditions than under steady pressure conditions over a 24-h lag before trials. The mean percentage response was greater under falling conditions than under rising conditions over a 12-h lag before trials. As in the 24-h results, the mean phototaxis response was greater under falling pressure conditions than under steady pressure conditions over a 9-h lag before trials.

Regressions of Psyllid Phototaxis on Pressure Trends, 3–48-h Before Trials. To consider the magnitude of pressure trend as a continuous variable in addition to its categories of falling, steady, and rising barometric conditions for the phototaxis bioassays in Table 3, the regression of overall response rate on pressure-trend magnitude was analyzed for each of the different 3–48-h time lags before trials. An analysis of covariance (ANCOVA) procedure in a generalized linear model framework (R Development Core Team 2008) was implemented to analyze the effects of pressure trend on response while allowing for differences

in mean response between light treatments and a common effect of pressure across all individuals:

$$Response [i, j] = \alpha[i] + \beta \text{ pressure} - trend \text{ magnitude}[j], \quad (1)$$

where $\alpha[i]$ is the mean percentage response in treatment i (1 of four different pattern contrasts), β is the coefficient of the effect of *pressure-trend magnitude* on mean response rate, and *pressure-trend magnitude*[j] indicates the difference in pressure for time lag j (either 3, 6, 9, 12, 24, 36, or 48 h before trial). Separate modeling was performed for each time lag. All of the regressions had significant F values, and the values of the pressure effects coefficient, β , were statistically significant except at 36- and 48-h time lags (Table 4). All of the estimates for β were negative; thus, both the regression and the categorical analysis methods suggest that falling pressure trends lead to increased percentages of phototaxis. The effects are statistically significant in both analyses for 9, 12, and 24 h, but the regression analysis suggests further that the effects remain significant at time lags as short as 6 and 3 h.

Discussion

Knowledge gained about environmental factors affecting behavior of an important pest such as *D. citri* has relevance in both fundamental and applied contexts. In this study, we found that large (>1 SD) changes in barometric pressure levels during the 24 h before testing are associated with significant differences in the mean percentages of *D. citri* responding in calling, mate-seeking, and phototaxis bioassays.

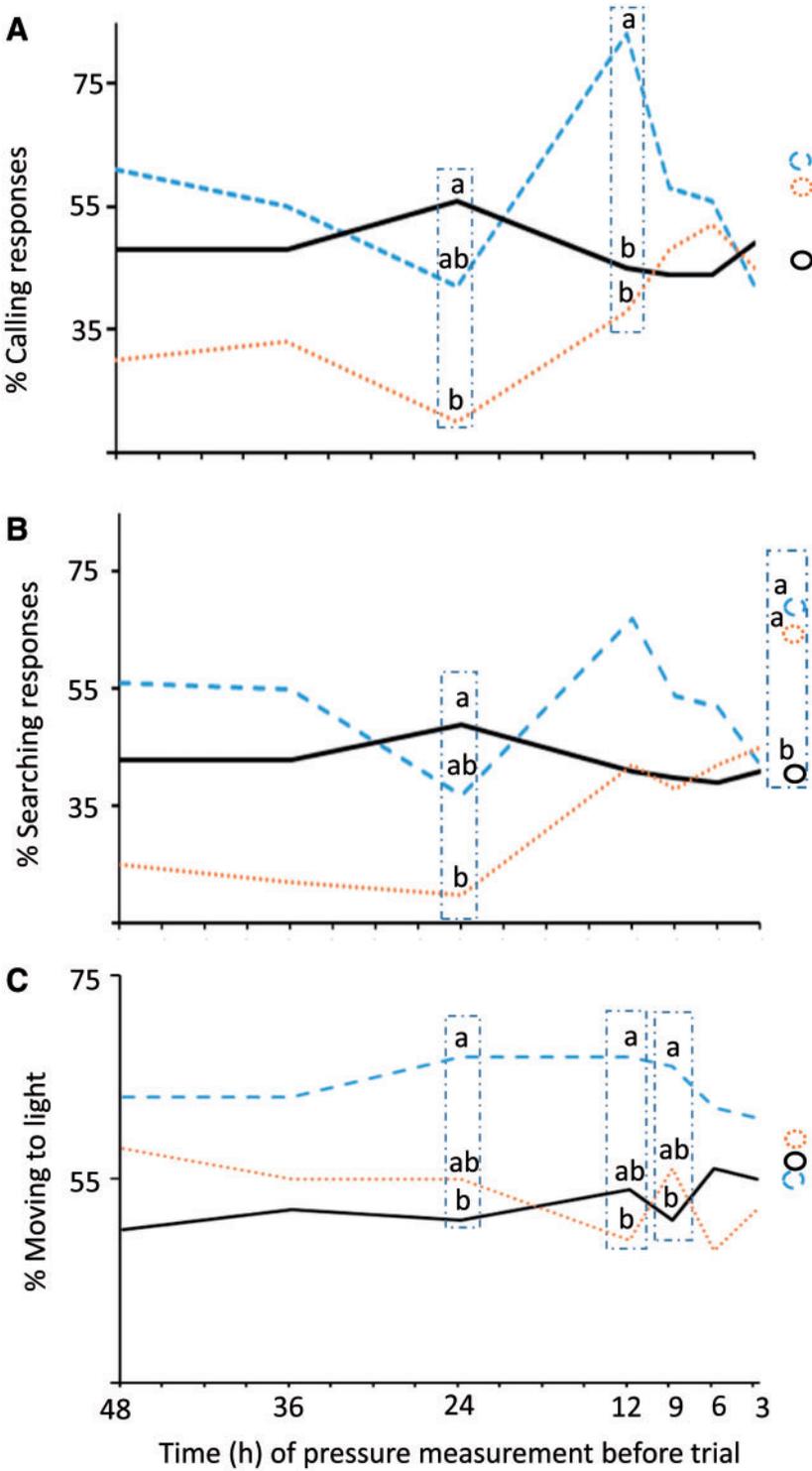


Fig. 2. Mean percentages of males performing A) calling, B) searching, or C) movement toward light under conditions of falling (dashed line), steady (solid line), or rising (dotted line) pressure trends, 3–48 h before trials. Circles indicate responses to low (dashed), steady (solid), or high (dotted) pressure at time of trial. Dash-dotted boxes designate contingency or analysis of variance tests where differences in calling, searching, or light responses of males exposed to falling (dashed line), steady (solid line), or rising (dotted line) pressure-trends or pressures were statistically significant (Table 2). Different letters designate means for each condition that were significantly different from each other by the binomial test for equal proportions (calling and searching) or Tukey’s HSD analysis (movement toward light).

Table 2. Contingency analysis of effects on calling and searching behavior of males exposed to falling, steady, or rising pressure or pressure-trend conditions measured at different time lags (h) before trials

Time before trial (h)	No. trials in condition category			Calling		Searching	
	Falling	Steady	Rising	χ^2		χ^2	
				χ^2	<i>P</i>	χ^2	<i>P</i>
0	20	92	19	4.696	0.09	8.92	0.01*
3	19	90	22	0.327	0.85	0.137	0.93
6	25	87	19	1.433	0.49	1.331	0.51
9	24	86	21	1.507	0.47	1.805	0.41
12	12	103	16	7.154	0.03*	3.820	0.15
24	19	87	25	10.520	0.005*	7.144	0.03*
36	20	93	18	1.928	0.38	4.317	0.12
48	18	93	20	3.823	0.15	3.770	0.15

The Pearson χ^2 tests for significant differences among response mean percentages for each condition (see Fig. 2), and *P* indicates the probability of obtaining by chance alone a value of χ^2 greater than computed.

Values of *P* < 0.05 are marked by asterisk.

Atmospheric pressure measurements are easily measured or retrieved from reliable sources, and the incorporation of such information into fundamental studies of *D. citri* behavior may provide insight into strategies that have evolved for optimization of reproduction and survival in different ecological contexts and, at the same time, may provide insight to help pest managers make decisions about the timing of control activities. We consider some potential insights below and conclude with discussion of other biologically relevant areas of atmospheric pressure research that remain unexplored.

Atmospheric Pressure Trends and Psyllid Mating Behavior. The observed reduction in *D. citri* mating activity after 24-h periods when pressure rose or fell by more than 1 SD is consistent with the hypothesis offered by Pellegrino et al. (2013) that lowered activity during weather extremes might reduce the risk of death from exposure to strong winds and rain. When compared with steady pressure conditions, a large increase in pressure was significantly associated with lower proportions of male psyllids calling and searching for females. Large decreases in pressure also were associated with lower proportions of response. Marchand and McNeil (2000) found a similar pattern when assessing effects of 24-h pressure changes on attraction of male *Aphidius nigripes* to female volatiles. In their study, steady pressure conditions resulted in the highest percentage of responsive males, while large increases in pressure resulted in the lowest. The mating system for *A. nigripes* is mediated by pheromone, while that of *D. citri* relies on vibrational cues, but engagement levels for the respective sexual behaviors were affected by pressure in a similar manner for both species. In other studies, the direction of the pressure trend was found to play an important role in behavioral responses (Lanier and Burns 1978, Pellegrino et al. 2013).

The survival context of the behavior being bioassayed also may be of significance in determining the magnitude and direction of changes in responsiveness. For example, given the increases in phototaxis that were

Table 3. Analysis of variance of effects on phototaxis of exposure to falling, steady, or rising barometric conditions measured at different time lags (h) before trials

Time before trial (h)	No. trials in condition category			Phototaxis	
	Falling	Steady	Rising	<i>F</i>	<i>P</i>
0	5	26	9	0.17	0.842
3	6	25	9	0.79	0.463
6	8	22	10	2.08	0.140
9	9	25	6	3.90	0.029*
12	7	25	8	3.54	0.039*
24	7	24	9	3.34	0.046*
36	20	22	9	1.96	0.156
48	10	21	9	3.07	0.059

Values of *P* < 0.05 are marked by asterisk.

Table 4. Regressions of mean phototaxis percentages on magnitudes of pressure trends over different time lags before trials

Time lag (h)	<i>F</i>	Adj <i>R</i> ²	β	SE	<i>t</i>	<i>P</i> > <i>t</i>
3	128.7	0.941	-1.804	± 0.702	-2.57	0.015*
6	125.1	0.939	-0.894	± 0.381	-2.34	0.025*
9	130.6	0.942	-0.817	± 0.305	-2.68	0.011*
12	133.5	0.943	-0.748	± 0.263	-2.84	0.007**
24	122.1	0.938	-0.44	± 0.206	-2.14	0.039*
36	11.7	0.933	-0.234	± 0.2	-1.18	0.247
48	107.2	0.93	-0.005	± 0.19	-0.03	0.979

All regressions were statistically significant at *P* < 0.01, with *F* statistics shown for 5 and 35 df, where $\beta \pm$ standard error (SE) in equation 1 estimates effect of pressure trend magnitude on mean response; values of *P* from Student's *t* test indicated that β was significantly different from 0 except for 36- and 48-h time lags.

Values of *P* < 0.05 are marked by asterisk, and values < 0.01 by double asterisk. Adj *R*² is the coefficient of determination, adjusted for the intercept.

observed for psyllids exposed to large pressure changes over 24 h, there may be survival benefits to focusing energy on long-distance dispersal or migration rather than on mating in such conditions.

In the case of vibrational communication, an alternative hypothesis can be considered that, in natural settings, it is reproductively advantageous for psyllids to engage less in vibrational communication when physical factors inhibit its effectiveness. Coccoft and Rodriguez (2005) characterized plant vibrations induced by wind as low-frequency patterns, while the frequencies produced by rain are much more variable and depend on the position of the droplet as it impacts the leaf. Both types of signals could overwhelm the low-amplitude, low-frequency vibrational calls of *D. citri*. In field trials, Tishechkin (2013) observed that small hemipterans emit signals during gaps between windy spells, perhaps because wind or other mechanical activities that induced interfering vibrations create conditions where substrate-borne communication becomes distorted and indistinguishable. Because the pressure changes that psyllids experience in the laboratory are not followed by disruptive weather factors such as precipitation, the reduced levels of mating behavior associated with such pressure changes may be an evolved rather than a learned response. Field studies on the vibrational communication behavior of *D. citri* in citrus

groves have begun only recently, and it is not yet known whether the observed effects of pressure trends on mating behavior in the laboratory are fully predictive of the effects that occur in field environments.

Technology for exploitation of vibrational signals holds promise for potential development of new methods in pest management (Eriksson et al. 2012, Mankin 2012). Consequently, it was of interest to us that the highest rates of psyllid mating responses were observed when pressure decreased significantly over a 12-hour period. This observation contrasted with the findings of Pellegrino et al. (2013), where gradual 12-h drops in pressure suppressed mating behavior in three unrelated insects. It is possible that the temporal pattern of the pressure change curve influences behavior; resolution of such effects would require experimental control over pressure trends. In addition, there could be important contextual differences in the two experiments that may have contributed to the contrasting findings. For example, Crespo and Castelo (2012) found that there was interaction between magnitude of pressure change and absolute pressure when they examined effects of pressure on olfactory orientation of *Mallophora ruficauda*. Also, given that there was an increased tendency of male psyllids to move to light after 12-h periods of falling pressure (Fig. 2C), there may have been a relatively greater fraction of psyllids interested in mate-seeking rather than flight during tests conducted after 12 h of falling barometer conditions compared with tests conducted under steady conditions. The experiments of Pellegrino et al. (2013) with *Diabrotica speciosa*, *Pseudaletia unipuncta*, and *Macrosiphum euphorbiae* may not have occurred in the same context of increased flight propensity under falling pressure conditions.

Atmospheric Pressure Trends and Psyllid Movement. Decreasing pressure conditions consistently were associated with higher proportions of *D. citri* hopping or flying to light in our study (Fig. 2C). This is consistent with findings by Wellington (1946), who showed that several dipteran species increased flight activity during controlled drops in pressure. Haufe (1954) described a pressure threshold for *Aedes aegypti*, above which flight activity was stimulated by falling pressure, but below which flight activity was stimulated by rising pressure. If such a pressure threshold exists for *D. citri*, it could provide an alternative framework for interpreting the effects of pressure on behavior.

In evaluating effects of atmospheric pressure on *D. citri* behavior, it may be important also to consider interactions between effects of humidity and pressure change. Water balance is generally a concern to small-bodied organisms, especially as they expend energy in flight. Air pressure experiments with *Aedes sp.* in an experimental chamber showed that very high humidity inhibited activity, even if the air pressure treatment itself had previously stimulated activity (Haufe 1964). In addition, one of the sensory mechanisms proposed by which insects detect pressure change is the use of an antennal hygroreceptor that responds to changes in both relative humidity and changes in air pressure (Tichy and Kallina 2010).

The occurrence of a 24-h period with large decreases in pressure is potentially an indicator of increasing *D. citri* populations in subsequent weeks, given that such decreases frequently are followed by rainfall within 12–24 h (Williams 1997, Burch 2013). Oviposition by *D. citri* females is strongly dependent on the availability of flush (Hall and Albrigo 2007), which is dependent on the age of the tree, pruning, storm damage, and environmental factors such as temperature and rainfall (Speigel-Roy and Goldschmidt 1996, Chen 1998).

The responsiveness of *D. citri* in phototaxis bioassays increased under 24-h periods with a rising pressure trend relative to response levels under steady conditions (Fig. 2C), although this trend was not statistically significant in our study. Often, rising pressure is indicative of dry sunny weather with low wind levels, so this laboratory observation is consistent with previous field observations that *D. citri* capture rates in sticky traps are greatest on windless sunny afternoons (Aubert and Hua 1990). This may be an example where an understanding of psyllid movement can be of importance for optimizing control strategies. In Florida, for example, psyllid scouting frequently is done on an area-wide basis and on relatively fixed time scales. Better estimation of when psyllids are most likely to move, perhaps through computer-generated-model predictions, may help improve the efficiency of scouting and trapping activities.

Challenges in Biologically Relevant Atmospheric Pressure Research. During this study, we adopted the hypothesis that small pressure changes are unlikely to affect behavior because even in absence of significant meteorological events, pressure fluctuates in a relatively predictable pattern semi-diurnally (Harris 1954). The results in Table 4 suggest, however, that some of the effects of atmospheric pressure trends on phototaxis can occur for even small pressure changes over short time lags. Indeed, many questions remain about the importance of the temporal pattern of the pressure change curve and the relevant starting and ending pressures during 12–24-h periods before the behaviors are observed. It should be noted also that this study was conducted in Florida, which frequently has convective rain not associated with standard weather fronts that are preceded by a clear pressure drop. Gaining a better understanding of the complex relationship between psyllid behavior and environmental factors of wind, rain, and atmospheric pressure (Burch 2009) will be important for future usage of barometric pressure cues as predictive tools for integrated pest management.

With many such challenges still to be addressed, the information gained about effects of atmospheric pressure trends on behavior in this study suggests that future investigations of *D. citri* behavior could benefit from including barometric pressure as a covariate in their statistical analyses. To confirm the results from this correlative study, we suggest future direct tests on the effects of pressure on mating or phototactic behaviors of *D. citri*. An example is the study of Jenkins et al. (2015), which indicates that *D. citri* abundance in Puerto Rico decreases with elevation. It is not yet

known whether direct effects of atmospheric pressure or other factors contributed to such a result.

Finally, it should be noted that there have been no investigations yet that demonstrate presence of atmospheric pressure sensory receptors in *D. citri*, although there is some evidence suggestive that this species has sensory receptors for pressure or humidity or both. For example, humidity changes already are known to affect *D. citri* behavior in field environments (Hall and Hentz 2011). In addition, sensory receptors have been identified that respond to both humidity and pressure changes in cockroaches and stick insects (Tichy and Kallina 2010). Further study is required to determine if such receptors occur also in *D. citri*, and whether they mediate sensation of barometric pressure changes. Indeed, if sets of differentially sensitive atmospheric pressure receptors are found in *D. citri* and other insects whose behaviors are affected by variations in atmospheric pressure, the finding could help investigators account for the many different types of behavioral responses to atmospheric pressure changes that were noted at the beginning of the discussion. Peripheral (Zhang et al. 2013) and central nervous system adaptive response mechanisms (McNamara et al. 2013) already have been confirmed for other insect sensory processes that result in a wide range of positive or negative behavioral responses to environmental stimuli of different magnitudes.

Alternatively, *D. citri* may sense instead a different cue that is correlated with barometric pressure change, such as the ratio of positive to negative ions (Pino and La Ragione 2013), which has been shown to affect human behavior.

Acknowledgments

We thank Betty Weaver, Heidi Burnside, and Everett Foreman (USDA-ARS-CMAVE) for rearing assistance and technical consultation. Funding was provided in part by a grant from the Citrus Research and Development Foundation. Mention of a trademark or proprietary product is solely for the purpose of providing specific information and does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable. The USDA is an equal-opportunity employer.

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Received 19 March 2015; accepted 30 June 2015.