1 **Sprinkler flow rate affects dairy cattle avoidance of spray to the head, but not overall, in an**
2 **aversion race**

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8 Abstract

9 Spraying cattle with water elicits behavioral responses ranging from willing use to avoidance of
10 wetting the head or entire body. This variation may be partly explained by sprinkler flow rate, as higher
11 flow rates result in greater heat abatement and spray impact. An aversion race can be used to evaluate the
12 emotional valence cows associate with treatments, based on the prediction that they will approach
13 negative (vs. positive) stimuli more slowly and require greater pressure from a handler. Our objective was
14 to evaluate how heat load affects the degree to which dairy cattle show reluctance to approach sprinklers
15 with different flow rates in an aversion race. We compared unsprayed treatments ($n = 8$ with feed, $n = 16$
16 without) to 0.4 L/min ($n = 14$) and 4.5 L/min ($n = 17$) sprinklers, which differed eight-fold in spray
17 impact (1.1 vs. 8.9 kPa , respectively). Over 10 trials, cows were required to approach treatments in a
18 covered, narrow raceway (average outdoor air temperature, mean $\pm$ SD, $31 \pm 3.8^\circ\text{C}$). We compared time
19 to complete the race (transit time), the pressure handlers applied, and head posture. Overall, handlers
20 applied pressure half as often when feed was offered but there were no other treatment differences in
21 handling pressure or transit time. Thus, we excluded the feed treatment when examining interactions with
22 heat load and sprinkler flow rate. In warmer weather, transit time increased (by 13 s per 10°C increase).
23 As their respiration rate increased, unsprayed cows moved more slowly (by 7 s per 10 breaths/min
24 increase), whereas sprayed cows did not. Handling pressure was not affected by heat load or spray
25 application, but head posture depended on sprinkler flow rate. When cows approached 4.5 L/min , they
26 lowered their heads nearly five times as often compared to 0.4 L/min or no spray (proportion of trials $\pm$
27 SE, 0.81 ± 0.05 , 0.17 ± 0.06 , and 0.15 ± 0.05 , respectively). This may have been an attempt to reduce
28 exposure of sensitive body parts to higher-impact spray. Indeed, when we applied von Frey
29 monofilaments to the ear and shoulder ($n = 56$), the ear had a lower response threshold (greater
30 sensitivity) than the shoulder [mean (95% confidence interval), 0.2 (0.1 to 0.4) vs. 1.2 (0.5 to 2.5) g of
31 force, respectively, back transformed from natural log values]. In conclusion, each measure provided
32 different insights about reluctance to approach treatments. Transit time increased in response to heat load
33 rather than aversion. Handling pressure reflected willingness, as it was needed less often when cows were

offered a feed reward, but this measure did not differ among sprinkler treatments. Therefore, although cows lowered their heads to avoid exposing sensitive areas to higher-impact spray, they did not show overall aversion to this type of spray.

Keywords: dairy cattle; aversion race; head posture; heat load; water spray; von Frey monofilaments

1. Introduction

In dairy cattle, heat load accumulation can result in increased body temperature, decreased milk yield (West, 2003) and fertility (De Rensis and Scaramuzzi, 2003), and in extreme cases, mortality (Stull et al., 2008; Morignat et al., 2014). For heat abatement in hot, dry climates, spraying cows with water is common (e.g., 62% of milking herds ≥ 500 head in the U.S.; USDA, 2010). In studies that provide spray, this strategy reduces body temperature, respiration rate, and air temperature (**T**) in the vicinity of sprinklers (Kendall et al., 2007; Chen et al., 2013) when compared to shade alone.

In freestalls and drylots that provide spray, sprinklers are typically mounted over the feeding area. In these settings, cattle can choose to stand beneath the spray, and thus their behavior moderates the degree of heat abatement received. Cows' behavioral responses to sprinklers vary across studies, ranging from voluntary spray usage (e.g., Chen et al., 2013) to avoidance of wetting the head (e.g., Schütz et al., 2011) or entire body (Marcillac-Embertson et al., 2009). Some of this behavioral variation may be explained by sprinkler flow rate, which determines both heat abatement and spray impact.

Higher flow rates (≥ 1.3 L/min, when applied for ≥ 3 min) provide greater heat abatement, both when cows are required to use spray (≥ 1.3 vs. 0.4 L/min applied intermittently during 1 h; Chen et al., 2015) and when they are given a choice (4.5 vs. 0.4 L/min for 12 min; Chen et al., In review). It is unknown whether cows choose to use spray on the basis of heat abatement efficacy, although it has been shown that they prefer shade that provides better protection from solar radiation (Schütz et al., 2009). If cattle indeed choose spray based on cooling effectiveness, they should prefer higher flow rates. In some studies with higher flow rates, cattle have indeed been found to prefer sprinklers over none (1.3 vs. 0

L/min, and 3.3 vs. 0 L/min, respectively; Parola et al., 2012; Chen et al., 2013), but not in others (2.6 vs. 0 L/min, and 4.5 vs. 0 L/min, respectively; Parola et al., 2012; Chen et al., In review). In addition, in one study, some cows rarely used a 7.3 L/min shower (i.e., <1 h/d; Legrand et al., 2011), and in another, heifers anecdotally avoided 30 L/min sprinklers altogether (Marcillac-Embertson et al., 2009). Nevertheless, although cows preferred sprinklers with a lower flow rate (0.4 L/min) compared to no spray in a 12-min paired comparison, they had no preference between 0.4 and 4.5 L/min sprinklers (Chen et al., In review). Therefore, the role of heat abatement efficacy in determining cattle use of higher or lower flow rates remains unclear.

Cattle may avoid higher flow rates in cooler weather to prevent heat loss. Cows tested when 24-h T averaged 18°C did not prefer sprinklers compared to shade or ambient conditions (Schütz et al., 2011). Physiological responses also indicate attempts to counteract heat loss: when cows were sprayed in the morning (average T, 24°C; Araki et al., 1985) or on days with T <23°C (Kendall et al., 2007), body temperature temporarily increased afterward. When T increased, however, so did the amount of time cows spent using 7.3 and 3.3 L/min spray (Legrand et al., 2011; Chen et al., 2013). Likewise, although cattle did not prefer 2.6 and 4.5 L/min sprinklers compared to shade across all testing conditions, when heat load increased their preference for spray became more marked (Parola et al., 2012; Chen et al., In review).

Cattle might also sometimes avoid higher flow rates because they deliver greater spray impact. The head may be particularly sensitive, and when standing under spray, cattle lower (Kendall et al., 2007; Tucker et al., 2007; Schütz et al., 2011) or keep their heads out of the spray radius (Schütz et al., 2011; Chen et al., 2013). To evaluate whether the head is more sensitive to mechanical stimulation compared to the trunk, von Frey monofilaments can be applied to elicit responses such as ear flicks (previously used in the context of disbudding in calves; Espinoza et al., 2013; Mintline et al., 2013) and skin twitches (e.g., on the withers of ponies; Lansade et al., 2008). Cows exhibited more skin twitches under spray in one study, perhaps in response to droplets (Schütz et al., 2011), but this was not replicated (Chen et al., In review). The effect of spray impact on head-specific avoidance has not been examined.

When cows do not prefer spray, the emotional valence underlying this choice is unknown. The

aversion race method has been used to evaluate emotional valence (reviewed by Rushen, 1996), and is based on the principle that animals move less willingly (more slowly, with more pressure from a handler, or both) toward aversive stimuli, which indicates negative emotional valence. Using this method, Pajor et al. (2000) demonstrated that dairy cows perceived being shouted at versus offered feed negatively and positively, respectively, relative to a silent handler without feed. These treatments could provide comparison points to evaluate the emotional valence cows associate with different spray flow rates.

Our objectives were to evaluate sensitivity to mechanical stimulation on the ear and shoulder, and to investigate three questions about spray flow rates: (1) what is the overall emotional valence cows associate with each, (2) how does this variable affect head-specific avoidance, and (3) how does heat load affect cows' willingness to approach each? We predicted the ear would be more sensitive than the shoulder, and higher flow rates would result in more head-specific spray avoidance. We predicted the emotional valence cows would associate with higher flow rates would be less positive than lower ones overall. Finally, we predicted cows would approach spray more willingly when heat load was higher, possibly changing the emotional valence associated with higher flow rates.

2. Materials and methods

2.1 Animals and housing

The study was conducted during summer (June to September 2013) at the University of California-Davis (UC Davis) dairy facility, with all procedures approved by the Institutional Animal Care and Use Committee. Fifty-eight lactating Holstein-Friesian dairy cows (40 pregnant) were selected based on average daily milk yield ≥ 34 kg, as metabolic heat production increases with milk yield. On average, the cows had daily milk yield (mean $\pm$ SD) of 40 ± 4.9 kg, parity of 2.0 ± 1.1 , days in milk of 184 ± 73 , and body weight of 701 ± 83 kg.

Cows were tested for eight days in nine cohorts (of five to seven cows), each housed in a group of 24 total (including those not being tested). The concrete-floored home pen included two water troughs (automatically refilled to 384 and 716 L, respectively) and 24 shaded, sand-bedded freestalls with two

overhead fans (36-DMCH; Future Products Corp., Mosinee, WI, USA). Cows were fed thrice daily during milking (05:00 and 17:00 h) and at 11:00 h with a total mixed ration (TMR) formulated to National Research Council (1989) requirements using the PC Dairy system (Bath and Strasser, 1990). The unshaded feed bunk was fitted with 13 soaker nozzles (TF-VP7.5 Turbo FloodJet wide angle flat spray tip, 4.9 L/min; Spraying Systems Co., Wheaton, IL, USA) that delivered 1.5 min of continuous spray, followed by 13 or 5 min off (at $T \geq 22.2$ or 29.4°C , respectively).

2.2 Procedures in the chute

On d 1 and 8 cows were moved to a chute for sensitivity testing and body temperature logger insertion and removal.

2.2.1 Von Frey monofilament testing

Two locations on the right side of the body were selected to represent the head and trunk: the back of the ear and the shoulder (Fig. 1), respectively. Behaviors used to assess responses to mechanical stimulation were an ear flick or a skin twitch on the shoulder. In order to ensure that cows responded to the testing stimuli rather than insects, debris was brushed from the hair coat on both sides of the body and a light coating of insect repellent (Prozap VIP; Neogen Corp., Lexington, KY, USA) was applied around (but not directly on) the testing sites with a washcloth. Sensitivity was assessed using a set of 20 von Frey monofilaments (0.008, 0.02, 0.04, 0.07, 0.16, 0.4, 0.6, 1, 1.4, 2, 4, 6, 8, 10, 15, 26, 60, 100, 180, and 300 g of force; Touch-Test Sensory Evaluators; North Coast Medical Inc., Gilroy, CA, USA), each mounted on a plastic handle. To test each body part, the tip of the 0.008 g monofilament was pressed against the hair coat; once the monofilament bent, it was held in place for 1 s. If a response was observed within 2 s after the monofilament was removed, the threshold was recorded; if not, the next level of force in the sequence was applied. The total time we allowed for a response (3 s) was based on the 3 to 4 s used to test the hind paw in rats (Vivancos et al., 2004) and 2 to 3 s near the tail in pigs (Sandercock et al., 2011). The order of testing the ear and shoulder was balanced among cows. In order to exclude trials in which cows responded to seeing the stimulus approaching, a sham assessment was performed before each filament was applied to the ear by bringing the other side of the handle close to this body part.

2.2.2 Body temperature loggers

Following sensitivity testing, data loggers (Minilog12-TX, accuracy: $\pm 0.1^{\circ}\text{C}$, resolution: $\pm 0.015^{\circ}\text{C}$, 1.6-cm diameter $\times$ 7-cm length; Vemco Ltd., Bedford, Nova Scotia, Canada) were inserted (d - 1) and removed (d 8). These were attached to shortened, hormone-free controlled internal drug release inserts (InterAg, Hamilton, New Zealand) to record intravaginal body temperature at 2-min intervals 24 h/d.

2.3 Aversion race testing

2.3.1 Treatments

Testing was conducted adjacent to the home pen. Portable livestock fencing (Powder River, Provo, UT) was used to construct a holding pen and a U-shaped aversion race (Fig. 2); the latter was in a solid-roofed area enclosed on three sides. The race had five segments delineated with painted white lines on the concrete floor: starting lane, first straightaway, bend at halfway, second straightaway, and treatment area. There were seven treatments (Table 1): three without spray (based on rewarding, neutral, and aversive treatments in Pajor et al., 2000) and four with spray applied at two different flow rates (used in Chen et al., In review). Spray was controlled manually with a ball valve, and was delivered at the end of the treatment area with a soaker nozzle (TK-FloodJet wide angle flat spray tip; Spraying Systems Co., Wheaton, IL, USA) mounted at 2.7-m-high and angled such that the spray wetted the entire floor of the treatment area.

To manipulate heat load, half of the sprayed cows had shade (DisplayShade, 500-denier polyester cover rated for 100% UV protection; Caravan Canopy International, La Mirada, CA, USA) over the holding pen, whereas the other half did not. All unsprayed cows had shade. Each cow spent 20 min in the holding pen, and one of 12 trained observers recorded respiration rate at the start and end of this period. Reliability was determined from 31 video clips. Pearson correlations for interobserver reliability for pairs of observers (each compared against J. M. Chen as the gold standard) ranged from $r = 0.994$ to 0.999 , and for intraobserver reliability ranged from $r = 0.993$ to 0.999 .

Cows were tested in cohorts, and were assigned to treatments randomly, within the constraint of balancing for milk yield and pregnancy status. A maximum of one cow per treatment was included in each of the nine cohorts for a total of seven to nine cows per treatment. Each cow was tested twice daily between 11:00 and 16:00 h on d 2 to 6 (10 trials total). Before each cow entered the race, the entire floor was wetted with a hose for consistency among treatments.

2.3.2 Race habituation

On d 1, cows were exposed to the holding pen (which had shade and a TMR-filled bucket) and empty aversion race. An experienced non-study cow (rewarded previously with feed) led individual naïve cows to complete the entire race at least once. To encourage forward movement, a handler followed but did not touch the naïve cow. If the naïve cow balked, the experienced cow was moved behind her.

2.3.3 Testing procedure

One of three handlers was consistently assigned to each cow for the aversion race. Once the cow was in the starting lane, the handler pulled a rope to open a wooden gate, commencing the test. Increasing levels of pressure were applied following a standardized scale (Table 2; Schwartzkopf-Genswein et al., 1997; Pajor et al., 2000), and the highest levels used until the cow entered each of the four segments (first straightaway, halfway bend, second straightaway, and treatment area) were summed to generate a total handling effort score for each trial. Once the cow entered the treatment area, the handler shut a wooden gate behind her, and one of three other consistently assigned experimenters administered treatment for 1 min. For the spray treatments, the nozzle was turned on before the cow entered the race.

2.3.4 Video observations

During testing, three video cameras (CVC-627B; Speco Technologies, Amityville, NY, USA) mounted at 2.8-m-high continuously recorded the starting lane, halfway bend, and treatment area. One observer used digital surveillance software (GeoVision Surveillance System V8.5; GeoVision, Taipei, Taiwan) to record transit time in the race, from the time when the door between the starting lane and first straightaway made contact with the fence until the cow's left hind foot (which was closest to the camera) crossed the painted line into the treatment area. Intraobserver reliability, as determined from 114 video

clips using Pearson correlation, was $r = 0.999$.

Another observer scored chin position to determine head posture when cows entered the treatment area. Each cow was observed from when her muzzle crossed the painted line into the treatment area until her left hind foot did. One-zero sampling (Martin and Bateson, 2007) was used to score chin position during this interval, which was on average (mean $\pm$ SD) 15 ± 17 s (range 2 to 134 s). If the poll was the forward-most part of the cow's body at any time during this interval, her chin was scored as tucked, and her head was thus considered lowered (Fig. 3). If the cow's muzzle was always forward-most, her head was scored as not lowered. Intraobserver reliability, as determined from 111 video clips using the coefficient of concordance (Hollenbeck, 1978; Martin and Bateson, 2007), was $\kappa = 0.838$.

2.4 Weather conditions

A portable weather station (WS-16; NovaLynx Corp., Auburn, CA, USA) placed approximately 55 m from the entrance of the race recorded T ($^{\circ}\text{C}$), black globe temperature (BGT, $^{\circ}\text{C}$), solar radiation (SR, W/m^2), relative humidity (RH, %), wind speed (WS, m/s), and rainfall (mm) at 5-min intervals. Additional measures of heat load were temperature-humidity index (THI) and heat load index (HLI), calculated according to the following equations from Kelly and Bond (1971) and Gaughan et al. (2008), respectively:

$$\text{THI} = (1.8 \times T + 32) - [(0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T - 26)]$$

$$\text{HLI} = \text{if } [\text{BGT} > 25, 8.62 + (0.38 \times \text{RH}) + (1.55 \times \text{BGT}) + \exp(-\text{WS} + 2.4) - 0.5 \times \text{WS}], \text{ else} \\ [10.66 + (0.28 \times \text{RH}) + (1.3 \times \text{BGT}) - \text{WS}]$$

2.5 Statistical analysis

Analyses were conducted using SAS software (Version 9.4; SAS Institute Inc., 2014). For sensitivity testing, data from 13 trials were excluded (two for cows removed during the study due to mastitis, and 11 in which cows responded with an ear flick during the sham assessment; final $n = 56$). For responses in the aversion race and pre-test holding pen, data from three cows were excluded (the two that developed mastitis, and one that refused to enter the testing area; final $n = 55$). All analyses were

conducted using PROC MIXED with cow as the experimental unit and using the Kenward-Roger degrees of freedom method (Gomez et al., 2005). Except where noted, all models with repeated measures included a REPEATED statement for trials within cow, and used a Toeplitz covariance structure (Littel et al., 2000).

2.5.1 Sensitivity to von Frey monofilaments

When cows did not respond to the highest level of force (300 g), the threshold for that body part was scored as 500 g, which represents the next step in the logarithmic series for increasing force. Scores for each body part were natural-log transformed to meet assumptions of normality and homoscedasticity. The amount of force needed to elicit a response was compared between the ear and shoulder using a model with fixed terms for test (d 1 and 8), body part order, and body part. The model also included a repeated term for test within cow, and used a compound-symmetry covariance structure.

2.5.2 Head posture

In order to evaluate head posture when cows approached the treatment area, treatments were grouped into three categories: no spray (n = 24), 0.4 L/min spray (n = 14), and 4.5 L/min spray (n = 17). The proportion of trials in which cows lowered their heads (out of 10) was compared using a model with a fixed term for treatment. Comparisons were conducted for all three pairs of treatments using the PDIFF function.

2.5.3 Pre-test shade and heat load

In order to determine whether shade over the holding pen reduced heat load, respiration rate was compared between cows that had shade (n = 40) and those that did not (n = 15) at the start and end of the 20-min holding period. Body temperature was also evaluated at those time points, as well as after cows completed the aversion race. Each time point was evaluated separately for respiration rate and body temperature using a model with a fixed term for shade.

2.5.4 Overall transit time and handling effort

To evaluate behavior when cows were naïve to the seven treatments (trial 1), time to complete the race (transit time) and summed handling effort score were examined separately using models with a fixed

term for treatment.

To evaluate the relative emotional valence of all seven treatments, transit time and handling effort were evaluated for the subsequent non-naïve trials (2 to 10) combined. A binary measure of handling effort was created by calculating the proportion of trials for which summed score >0. Average transit time and the proportion of trials in which handling pressure were applied were evaluated separately using a model with a fixed term for treatment. When there was a significant ($P < 0.05$) effect of treatment, comparisons were conducted between all pairs of treatments using a Tukey-Kramer adjustment (Stoline, 1981).

2.5.5 Effects of heat load on transit time and handling effort

Based on the results of the analysis in section 2.5.4, the feed treatment was excluded from comparisons to evaluate the effects of heat load (regardless of pre-test shade) on cattle willingness to approach different flow rates in the non-naïve trials (2 to 10). The remaining treatments were grouped into three categories: no spray (n = 16), 0.4 L/min (n = 14), and 4.5 L/min (n = 17). The model included fixed terms for treatment, heat load (respiration rate, body temperature, T, THI, HLI, or BGT), and the treatment × heat load interaction. This analysis was repeated separately for transit time and summed handling effort score, and for each measure of heat load. For HLI, data were excluded for 3 d for which WS measurements were unavailable. Parameter estimates were determined using the ESTIMATE statement, including whether the relationship between heat load and transit time or summed handling effort score had a slope that was significantly different from zero.

3. Results

3.1 Environmental conditions

Weather conditions are summarized in Table 3. Rainfall was 0 mm throughout the study.

3.2 Sensitivity to von Frey monofilaments

The ear had a lower response threshold than the shoulder (ear vs. shoulder, 0.2 vs. 1.2 g of force, respectively, back transformed from natural log values; 95% confidence intervals, 0.1 to 0.4 g vs. 0.5 to

2.5 g of force, respectively; $F_{1,149} = 23.6$, $P < 0.001$). When the lowest level of force (0.008 g) was applied, 16 cows responded with an ear flick, and 10 responded with a skin twitch on the shoulder. The highest level of force (300 g) was reached without observing an ear flick or skin twitch on the shoulder for four and nine cows, respectively.

3.3 Head posture

Cows lowered their heads nearly five times as often when approaching 4.5 L/min spray compared to 0.4 L/min and 0 L/min, respectively (overall: $F_{2,52} = 49.8$, $P < 0.001$; pairwise comparisons with 4.5 L/min: $P < 0.001$; 0 vs. 0.4 L/min: $P = 0.735$; Fig. 3).

3.4 Pre-test shade and heat load

Compared to cows that did not have pre-test shade, this resource resulted in lower respiration rate when cows were released from the holding pen ($F_{1,52} = 4.7$, $P = 0.035$; Fig. 4A). Shade also tended to result in lower body temperature at that time ($F_{1,51.9} = 3.1$, $P = 0.087$; Fig. 4B), as well as after cows completed the aversion race ($F_{1,51.8} = 3.5$, $P = 0.069$). When cows first entered the holding pen, starting respiration rate ($F_{1,52.9} = 2.3$, $P = 0.137$) and body temperature ($F_{1,52.1} = 0.9$, $P = 0.348$) did not differ between shade treatments.

3.5 Overall transit time and handling effort

When cows were naïve to treatments (trial 1), there were no differences in transit time or handling effort ($F_{6,48} \leq 1.4$, $P \geq 0.238$). The proportion of subsequent trials in which handlers used pressure (binary outcome) was nearly halved when feed was offered compared to the other treatments (overall: $F_{6,48} = 4.4$, $P = 0.001$; feed vs. control: $P = 0.067$; all other pairwise comparisons with feed: $P \leq 0.040$; all non-feed pairwise treatment comparisons: $P \geq 0.725$; Fig. 5A). There were no overall differences among treatments in transit time ($F_{6,48} = 1.0$, $P = 0.424$; Fig. 5B).

3.6 Effects of heat load on transit time and handling effort

There was a tendency for a treatment $\times$ respiration rate interaction ($F_{2,339} = 2.9$, $P = 0.057$; Fig. 6). As respiration rate increased, cows approached the no-spray treatments more slowly (slope estimate = 7 s per 10 breaths/min increase, $F_{1,364} = 2.4$, $P = 0.017$). For 0.4 and 4.5 L/min spray, respiration rate did

not affect time to complete the race (slope estimates not significantly different from zero: $F_{1,286 \text{ to } 339} \leq 1.1$, $P \geq 0.283$). As T or THI increased, cows approached the treatment area more slowly (slope estimates, 13 and 18 s per 10°C and 10-unit increase in T and THI, respectively, $F_{1,223 \text{ to } 268} \geq 4.1$, $P \leq 0.043$), regardless of treatment (no treatment $\times$ T or THI interactions: $F_{1,222 \text{ to } 268} \leq 0.5$, $P \geq 0.623$).

Body temperature, HLI, and BGT did not affect transit time ($F_{1,288 \text{ to } 365} \leq 2.0$, $P \geq 0.160$), regardless of spray flow rate (no treatment $\times$ body temperature, HLI, or BGT interactions: $F_{2,286 \text{ to } 358} \leq 0.6$, $P \geq 0.574$). For handling effort, there were no main effects of heat load (respiration rate, body temperature, T, THI, HLI, or BGT: $F_{2,250 \text{ to } 381} \leq 2.3$, $P \geq 0.129$), regardless of spray flow rate (no treatment $\times$ respiration rate, body temperature, T, THI, HLI, or BGT interactions: $F_{2,249 \text{ to } 376} \leq 1.3$, $P \geq 0.264$).

4. Discussion

Previous studies found that cows sometimes willingly use sprinklers, but other times avoid spray to the head or entire body. We evaluated the effects of sprinkler flow rate on cattle willingness to approach spray in an aversion race. Transit time, handling effort, and head posture provided different insights about behavioral responses to spray. All cows took longer to complete the race in warmer weather overall, and unsprayed cows did so as their respiration rate increased. As handling effort did not show these patterns, slower movement was likely a response to increased heat load rather than an indicator of reluctance to approach treatments. Sprinklers mitigated this heat related slowing: transit time did not increase with respiration rate when cows were offered 0.4 or 4.5 L/min spray. When cows encountered 4.5 L/min spray, they lowered their heads nearly five times as often compared to 0.4 L/min or no spray, perhaps to reduce exposure of sensitive areas to higher spray impact. Consistent with this idea, the ear had a lower response threshold (greater sensitivity) to mechanical stimulation by von Frey monofilaments.

4.1 Transit time and handling effort to approach treatments

In previous studies, greater transit time and handling effort both indicated reluctance to approach aversive stimuli. When measured on its own, transit time was greater in aversive compared to control treatments for laboratory rats (Karsh, 1962; Anderson et al., 1967), dairy cattle (Pascoe and McDonnell,

1986), sheep (Hargreaves and Hutson, 1990), and red deer in one study (Pollard et al., 1994), although not in another (Grigor et al., 1998). Both total transit time and the time handlers spent pushing sheep were greater in aversive compared to control treatments (Rushen, 1986; Rushen and Congdon, 1986). Likewise, for cattle, the number of times handlers prodded cows (with a plastic pipe; Arnold et al., 2008) and the magnitude of handling effort (Goonewardene et al., 1999; Pajor et al., 2000) distinguished between aversive and control treatments in the same manner as transit time. Only one study found no treatment differences for transit time or handling effort, perhaps because all treatments involved restraint (Schwartzkopf-Genswein et al., 1997).

Conversely, lower transit time and handling effort both reflect increased willingness to approach rewarding treatments, as shown by both measures when feed was offered in previous studies on cattle (Pajor et al., 2000) and sheep (Hutson, 1985). Likewise, in our study, handlers applied pressure half as often when feed was offered compared to when it was not, indicating it was more rewarding than the other treatments. However, we found no other overall treatment differences, and thus did not replicate the finding by Pajor et al. (2000) that shouting is aversive to dairy cattle. The shouting handler in the earlier study moved repeatedly between the front and side of each cow, an action that perhaps elicited additional fear (Waiblinger et al., 2006). Other relevant differences may have included the shouting volumes (decibel levels were not recorded in either study) or the degree of cattle habituation to similar sounds during regular handling (Arnold et al., 2007). Overall, we could not determine whether the emotional valence cattle associated with different spray flow rates was negative, or merely less positive than that associated with feed.

In this study, greater transit time did not indicate reluctance to approach treatments. Rather, moving more slowly seemed to be a direct response to increased heat load. Whereas speed to approach treatments was on average 0.5 to 0.7 m/s in other studies (weather conditions not reported; Schwartzkopf-Genswein et al., 1997; Goonewardene et al., 1999), in our study, average speed was 0.1 m/s for all non-feed treatments (based on 136 s average transit time). Cows took longer to complete the race overall in warmer weather (by 13 and 18 s per 10°C and 10-unit increase in T and THI, respectively), and as

respiration rate increased in the no-spray treatments (by 7 s for each 10 breaths/min increase), but did not require greater handling effort as heat load increased. Cows may have walked more slowly because physical activity increases heat load (body temperature increased 0.3°C in beef cattle moved 150 m in summer; Mader et al., 2005).

We initially predicted cows would approach spray more willingly as heat load increased. Rather, sprinklers mitigated the slowing associated with heat load: transit time did not increase with respiration rate for the 0.4 and 4.5 L/min spray treatments, in contrast with the no-spray treatments. Although we could not determine the degree of heat abatement that resulted from 1 min of spray, we previously found respiration rate was reduced relative to no spray when ≥ 0.4 L/min was applied for 12 min (either cumulatively over 1 h or continuously in Chen et al., 2015; Chen et al., In review, respectively), or when ≥ 4.5 L/min was sprayed for 3 min (Chen et al., 2015). The effect of sprinklers on transit time may have been apparent for respiration rate but not weather because physiological responses to the latter vary in degree, and the former measure directly reflects individual cows' attempts to reduce heat load (Eigenberg et al., 2000).

To our knowledge, no previous aversion race studies were conducted in warm weather. In addition to conducting the study in summer, we walked the cows from their home pen to the aversion race for testing. This walking may partly explain why respiration rate was 32 to 53% higher than in our previous studies in similar weather conditions, when cows remained in their pens with spray access (53 breaths/min from 11:00 to 18:00 h, and 49 breaths/min from 10:00 to 18:00 h, respectively; Legrand et al., 2011; Chen et al., 2013). Walking more slowly in the race was likely a response to avoid heat gain. Indeed, lethargy in summer is thought to explain reduced estrous behavior (De Silva et al., 1981; Gwazdauskas et al., 1983; Pennington et al., 1985). Our findings demonstrate that when heat load is high, transit time, in contrast with handling effort, does not distinguish between treatments that differ in emotional valence. Future studies should consider heat load before including transit time as a measure of reluctance.

4.2 Head posture

When cows stood under spray or rain in previous studies they avoided wetting their heads either by lowering (chin below brisket level; Kendall et al., 2007; Tucker et al., 2007; Schütz et al., 2011) or keeping their heads out of the spray radius (Schütz et al., 2011; Chen et al., 2013). The current study is the first to examine head specific spray avoidance when cows approach sprinklers, and the effect of flow rate on this behavior. As higher flow rates generate greater spray impact (e.g., nine-fold difference in theoretical total spray impact, F_i , between the spray treatments in this study), we predicted this would result in greater avoidance of wetting the head relative to less or no spray. Consistent with this idea, cows lowered their heads over 80% of the time when approaching 4.5 L/min (F_i 1.1 kPa) compared to less than 20% of the time for 0.4 L/min (F_i 8.9 kPa) or no spray.

This change in head posture appeared to be a transient response to spray, whereas in other contexts lowered head or neck carriage has been interpreted to indicate states of pain (head below brisket or withers, respectively; Petherick et al., 2013; Gleerup et al., 2015). Previously, we speculated such behavior in the context of sprinklers may reflect aversion toward exposing the head to spray (Kendall et al., 2007). However, despite the nearly five-fold greater frequency of head lowering we observed for 4.5 compared to 0.4 L/min spray, there was no difference in overall willingness to approach these options, as measured by handling effort. Likewise, in another recent study cows showed no preference between these options (12 min of spray; Chen et al., In review). Therefore, although cattle lower their heads in response to a higher flow rate, this may not translate into overall reluctance to approach spray delivered in this manner.

4.3 Sensitivity to von Frey monofilaments

Cows may have lowered their heads when approaching 4.5 L/min in an attempt to reduce exposure of sensitive areas to higher impact spray. Consistent with the head having greater sensitivity, the mean level of force to elicit a response was 83% lower on the ear than the shoulder (0.2 vs. 1.2 g, respectively). Likewise, in humans the forehead has a lower response threshold to von Frey monofilaments than areas on the arm or leg (Ackerley et al., 2014). To our knowledge, our study is the first to find differences in non-nociceptive sensitivity between body parts in cattle. For calves, although

the head was more responsive than the rump when 10 and 300 g of force were applied (Espinoza et al., 2013), the difference in that study likely reflected pain related to disbudding.

The higher average response threshold on the shoulder could also be due to this body part responding more slowly than the 3 s we allowed. However, this is unlikely, as skin twitches (i.e., the cutaneous trunci or panniculus reflex; DeLahunta and Glass, 2009) occur rapidly following stimulation (20 ms in guinea pigs; Blight et al., 1990). For ear flicks, we had concerns about visual stimuli eliciting this reaction, and discarded 11 trials (for both body parts) in which cows responded to a sham assessment. For the remaining trials, we found no effects of body part testing order, and thus no evidence that the sham assessment sensitized – or desensitized – cows to mechanical stimulation on the ear. It is unknown how the response thresholds to von Frey monofilaments correspond to spray impact. Under sprinklers, more skin twitches were observed in one study (Schütz et al., 2011), perhaps in response to droplets, but fewer were seen in another (Chen et al., In review), as spray may deter insects. Quantifying spray impact and insect load would help explain these differences.

5. Conclusions

The responses we measured provided insights about cows' reluctance to approach treatments. In previous studies, increased transit time and handling pressure both indicated reluctance to approach aversive stimuli in a raceway. Our study is the first to be conducted in warm conditions, and in this context, transit time increased in response to heat load, rather than aversion. Handling pressure reflected willingness to complete the race, as it was needed less often when cows were offered a feed reward. However, this measure did not differ among spray treatments. Therefore, although cows lowered their heads to protect sensitive areas from 4.5 L/min spray, they did not find higher impact spray aversive overall.

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Fig. 1. Two parts on the right side of the body of restrained dairy cows ($n = 56$) were evaluated for sensitivity using von Frey monofilaments: (A) the back of the ear, and (B) 12 cm from the point of the shoulder, along the infraspinatus muscle.

Fig. 2. Dimensions (diagram not to scale) of the shaded aversion race in which dairy cows ($n = 55$) were tested and the pre-test holding pen. Dashed lines indicate white paint on the concrete floor of the race that demarcated five segments: the (a) starting lane, (b) first straightaway, (c) bend at halfway, (d) second straightaway, and (e) treatment area (with an overhead spray nozzle). A handler opened the gate between segments (a) and (b) at the start of each test and closed another gate behind the cow when she reached segment (e). Cows could not access the blackened areas.

Fig. 3. Head posture was evaluated when cows entered the treatment area. (A) The head was scored as lowered if the poll was the forward-most part (1); otherwise, if the muzzle was always forward (2), the head was scored as not lowered. (B) The proportion of trials (out of 10/cow) $\pm$ SE in which cows showed lowered head posture when there was no spray ($n = 24$), 0.4 L/min ($n = 14$), or 4.5 L/min spray ($n = 17$); letters indicate differences among spray flow rates ($P < 0.001$).

Fig. 4. Mean $\pm$ SE for (A) respiration rate and (B) body temperature at three time points: when cows entered the holding pen, left it 20 min later, and completed an aversion race afterward. When cows were in the holding pen, $n = 40$ received shade whereas $n = 15$ did not. Differences in responses between shade and no-shade treatments are indicated at each time point: * $P < 0.05$; † $P < 0.09$.

Fig. 5. The (A) proportion of trials $\pm$ SE in which handlers applied pressure (score >0 ; Table 2) to move cows forward, and (B) average transit time $\pm$ SE to complete the aversion race. The initial trial was excluded, and treatments ($n = 7$ to 9 each) were compared for the subsequent non-naïve trials (2 to 10). Pairwise differences between the feed treatment and the others are indicated: ** $P < 0.01$; * $P < 0.05$; † $P < 0.07$.

Fig. 6. Fitted linear relationships for the effect of respiration rate on transit time for cows to approach one of three treatments at the end of an aversion race: no spray (and no feed; $n = 16$), 0.4 L/min ($n = 14$), or 4.5 L/min spray ($n = 17$). Treatment with slope that differs from zero is indicated: * $P < 0.05$.

Table 1. Seven 1-min treatments assigned to evaluate dairy cattle willingness to approach in a race

Treatment	Shade ¹	n ²	Description ³
Feed	Yes	8	Handler silently held a bucket with feed (approximately 1 kg of total mixed ration) at hip level
Control	Yes	8	Handler stood silently with hands in pockets
Shout	Yes	8	Handler shouted repeatedly (“hey,” “go,” and “come on”) with hands in pockets
0.4 L/min spray	Yes	7	TK-0.75 nozzle, operating pressure 138 kPa (1.4 kg/cm ²), theoretical total spray impact ⁴ F _i 1.1 kPa, average droplet size ⁵ 370 µm; handler stood silently with hands in pockets
	No	7	
4.5 L/min spray	Yes	9	TK-12 nozzle, operating pressure 69 kPa (0.07 kg/cm ²), F _i 8.9 kPa, average droplet size ⁶ 975 µm; handler stood silently with hands in pockets
	No	8	

¹Indicates whether cows had shade when held in a pen for 20 min before testing.

²Number of cows receiving each treatment (1 treatment/cow, repeated twice daily for 5 d).

³In all treatments, a handler stood at the end of the treatment area facing the cow.

⁴ $F_i = Q \times \sqrt{P} \times K$, where Q is the volumetric flow rate in L/min, P is the liquid operating pressure in kg/cm², and K is the constant 2.354 to convert to kPa (Spraying Systems Co., 2015).

⁵Spray Analysis and Research Services (1995).

⁶Spray Analysis and Research Services (1993).

Table 2. Handling effort scale¹ used to move dairy cows into each of four segments of an aversion race

Score	Description
0	If cow moves forward on her own within 30 s, apply no pressure
1	Whistle, use gentle vocal encouragement, and raise arm behind cow
2	Whistle, use gentle vocal encouragement, and prod cow's rump with fingers
3	Whistle, use gentle vocal encouragement, and use plastic tip of mechanical pencil to scratch cow's rump
4	Whistle, use loud vocal encouragement, and use plastic tip of mechanical pencil to prod cow's rump
5	Use loud vocal encouragement and push cow from behind with both hands
6	Use loud vocal encouragement and push cow from behind with one hand while grasping tail with the other

¹When scores 1 to 5 are reached, each step is repeated up to three times, until the cow moves; if the cow does not move after three repetitions, the handler escalates to the next score.

Table 3. Summary of daily weather conditions on experimental days in June through September 2013

Weather variable	Testing period ¹			24 h ²		
	Mean	SD	Range	Mean	SD	Range
Air temperature (T, °C)	31.0	3.8	21.3 – 43.5	23.8	3.4	19.0 – 32.2
Temperature Humidity Index (THI)	76	4	67 – 87	69	4	63 – 78
Heat Load Index (HLI) ³	87	5	70 – 104	68	5	61 – 88
Black globe temperature (BGT, °C)	41.3	3.9	29.8 – 55.2	28.2	3.4	22.9 – 36.1
Solar radiation (SR, W/m ²)	788	70	173 – 1102	285	37	174 – 327
Relative humidity (RH, %)	32	6	11 – 58	52	7	38 – 64
Wind speed (WS, m/s) ³	1.5	0.5	0.2 – 3.4	1.3	0.3	0.8 – 2.0

¹Means, SD, and range (minimum to maximum of observed values) are given for 11:00 to 16:00 h daily, when cows (n = 55) were tested.

²Means, SD, and range (minimum to maximum of daily mean values) are given for 24 h.

³Data for WS (and thus HLI) were unavailable for 3 d.

Chen et al. Fig. 1

Chen et al. Fig. 2

Chen et al. Fig. 3

(A) Head posture

(B) Proportion of trials with head lowered

Chen et al. Fig. 4

Chen et al. Fig. 5

(A) Proportion of trials with handling pressure applied

(B) Average transit time (s)

Chen et al. Fig. 6