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Seasonal, Landscape, and Attractant Effects on Lesser Grain Borer, *Rhyzopertha dominica* (F.), Captures in Northeast Kansas

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Abstract: The lesser grain borer, *Rhyzopertha dominica* (F.), is a highly diverse feeder and widely distributed throughout the United States in agricultural and non-agricultural landscapes. Six four-funnel Lindgren traps were deployed in feed mill, grain elevator, and native prairie landscapes, to determine the most attractive food and pheromone combination (attractant) and patterns in seasonal captures. Traps were baited with combinations of wheat (crimped, high moisture, pre-fed) with or without an *R. dominica* specific aggregation pheromone in 2017 and 2018. Traps were deployed for 48 h, collected, and the number of *R. dominica* counted. *Rhyzopertha dominica* was captured among all landscapes with all attractants. There was a significant correlation between temperature and *R. dominica* captures, with peak captures occurring during the warmest months. Significantly more *R. dominica* adults were captured in traps containing the pheromone. In 2017, pheromone traps captured 818% more *R. dominica* and 543% more than in 2018. The pheromone component in the trap was more attractive than any natural stored wheat condition and should be included in future studies. Understanding the seasonal patterns and changes in capture rates in agricultural and non-agricultural landscapes may be useful in determining times of increased immigration pressure into the newly harvested grain.

Keywords: lesser grain borer; seasonal captures; dispersal; native prairie; trapping; monitoring; pheromone; stored product insect

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1. Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.), is a serious pest of stored grain, especially wheat, throughout much of the world. *Rhyzopertha dominica* has been reported feeding on highly diverse food groups including seeds, turnips, various woods, beans, roots, and tubers [1]. This species is not known to infest grain in the field before harvest but can infest newly-harvested grain after it is loaded into farm bins or elevator silos [2]. Adults are strong flyers and have been captured in traps as far as 1.6 km from a centralized release point [3]. *Rhyzopertha dominica* is an internal feeder and only the adults can be observed in infested grain, which makes their detection difficult compared to external feeders in which all life stages are present and visible outside of the grain kernel. Larvae of *R. dominica* develop inside the grain kernel and feed on the germ and endosperm. Newly emerged adults will chew their way out of the kernel and continue to feed and cause economic losses in stored grain.

Upon locating and feeding on a suitable food source, *R. dominica* males release two aggregation pheromones, (*S*)-(+)-1-methylbutyl (*E*)-2-methyl-2-pentenoate (designated dominicalure-1, DL-1) and (*S*)-(+)-1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate (designated dominicalure-2, DL-2), which will attract more males and females and increase the infestation [4]. The minimum and maximum temperature limits for *R. dominica* development are 18.2 and 39 °C, respectively, at 70% r.h. on 14% moisture content (m.c.) wheat [5].

The limits on adult *R. dominica* flight activity are estimated to be between 16–20 °C and 37–44 °C under laboratory conditions [6–8]. However, *R. dominica* flight has been recorded in the United States (US) State of Oklahoma in mid-March, when the daily high temperature was approximately 11 °C [9]. This suggests that differences among strains or microclimate effects may result in field monitoring of *R. dominica* having a lower threshold for flight activity compared to laboratory colonies. There are two major peaks in daily flight activity of *R. dominica*. The first can be observed near sunrise and the second peak occurs in 2–3 h before sunset, and some flight occurs during the night [7,10,11]. The frequency of flight initiation is influenced by insect age [12,13], density and frass accumulation [14,15], temperature and humidity [13], light intensity [13], food deprivation [11,16], but not by sex of the beetles [12,13]. In the US at latitudes above 33° (e.g., States of Oklahoma, Kansas, and Nebraska), there can be three distinct seasonal peaks of *R. dominica* flight: first seasonal peak observed during early-spring or mid-March to early April (Spring), the second peak in August (Summer), and third peak in October/November (Autumn) [9,17]. However, at warmer locations, such as in the southeastern US, *R. dominica* may be captured routinely all year long [18].

Understanding the seasonal activity of *R. dominica* in and around areas of grain storage is important for pest management decisions to prevent and manage infestations. Over the years there have been multiple studies published on the flight activity, spatial and temporal distribution, dispersal, and feeding behavior of *R. dominica* near grain storage, open fields, and woodland forests throughout the world including Australia [19–21], Canada [22], India [23], Italy [24], Arkansas, Kansas, Oklahoma, and South Carolina in the United States [3,9,17,25,26]. *Rhyzopertha dominica* can be consistently captured using pheromone baited traps many kilometers away from the stored grain suggesting that *R. dominica* may be migrating between the natural habitats to grain stores or using the natural landscapes or wooded areas to either overwinter or maintain a continuous population [3,9,20,26,27]. Mark-recapture studies were undertaken using *R. dominica* adults released on a natural prairie landscape in northeast Kansas, and adults dispersed about 380 m on average and with maximum dispersal of more than 1000 m, which was the limit of the study [26]. In Oklahoma, *R. dominica* adults were captured as far as 1.6 km from the release point [3].

Often more *R. dominica* adults are captured in trap locations near woodlands in Spring and Autumn [26], which suggests that the woodland areas may serve as an overwintering site. At least 53 different plant species have been reported as potential hosts for *R. dominica* [28]. *Rhyzopertha dominica* can survive and reproduce on a variety of tree seeds and acorns of multiple oak species [29], cache materials found in eastern woodrat nests [30], and potato tubers and acorns [31], all of which can be found in natural environments outside of storage facilities. Both males and females will respond to plant volatiles of wheat, peanut, and cowpeas seeds, potato tubers, oak seeds, and woody tissues of the eastern red cedar and loblolly pine [31]. However, when plant volatiles alone were used as bait in Lindgren traps, no adult *R. dominica* were captured [31]. When Lindgren traps baited with the plant material and live adult males feeding on the plants, the mean number of *R. dominica* captures ranged from 0.5–6 beetles/trap [31]. Therefore, the male-produced aggregation of pheromone from feeding could be the most important factor in attracting conspecific males and females to a suitable food source compared to the suitability of the food source for reproduction.

It is not well understood how *R. dominica* locates a potential food source, whether it is by chance or by olfactory-guided mechanisms. Numerous studies have been conducted on the role of plant volatiles on *R. dominica* walking responses over short-range laboratory conditions, but there is no consensus on the role of plant volatiles on host-location behavior. To understand how *R. dominica* responds to food and pheromone volatiles in a broader field setting and how this might impact our ability to improve detection, we evaluated six different wheat and pheromone attractant combinations at three different locations, native prairie, feed mill, and grain elevator in Northeast Kansas, and evaluated their influence on overall *R. dominica* captures during two field seasons. An understanding of

temporal, seasonal, and food/pheromone attractiveness at each location will provide a deeper understanding of the biological preference of *R. dominica* and an insight into their host selection preference in three distinctly different ecological locations.

2. Materials and Methods

2.1. Study Sites

There were three trapping sites in Northeast Kansas selected for this study that represented landscapes around a food/feed processing facility, a grain storage elevator, and native prairie grasses (Table 1). Each site contained six Lindgren traps (described later) placed in a transect approximately 10–12 m apart. The first and last trap locations are listed in Table 1. The approximate distances between each field site location are depicted in Figure 1.

Table 1. Description and location (latitude and longitude of the traps at each end of the transect) of field sites.

Field Site Location			Site Description
Konza Prairie Biological Research Station (Native Prairie)	Trap 1	39°5′30.6′′ N; 96°35′15.0′′ W	3487 ha native tallgrass prairie preserve located in the Flint Hills region of Northeast Kansas. Areas, where traps were placed, are grazed by bison and burned yearly. Traps were placed along a dirt roadway near low underbrush.
	Trap 6	39°5′28.9′′ N; 96°35′13.5′′ W	
KSU Feed Mill (Feed Mill)	Trap 1	39°12′23.8′′ N; 96°35′25.0′′ W	A pilot-scale feed mill was used for training, extension programs, and which produced small lots of animal feed. Traps were placed on the north side of the feed mill near the underbrush.
	Trap 6	39°12′25.4′′ N; 96°35′23.5′′ W	
Commercial Grain CO-OP Elevator (Grain Elevator)	Trap 1	39°11′37.0′′ N; 96°30′12.3′′ W	Commercial grain cooperative (CO-OP) grain elevator located in Northeast Kansas. Traps were placed between concrete storage silos and an active railroad line.
	Trap 6	39°11′36.7′′ N; 96°30′14.3′′ W	

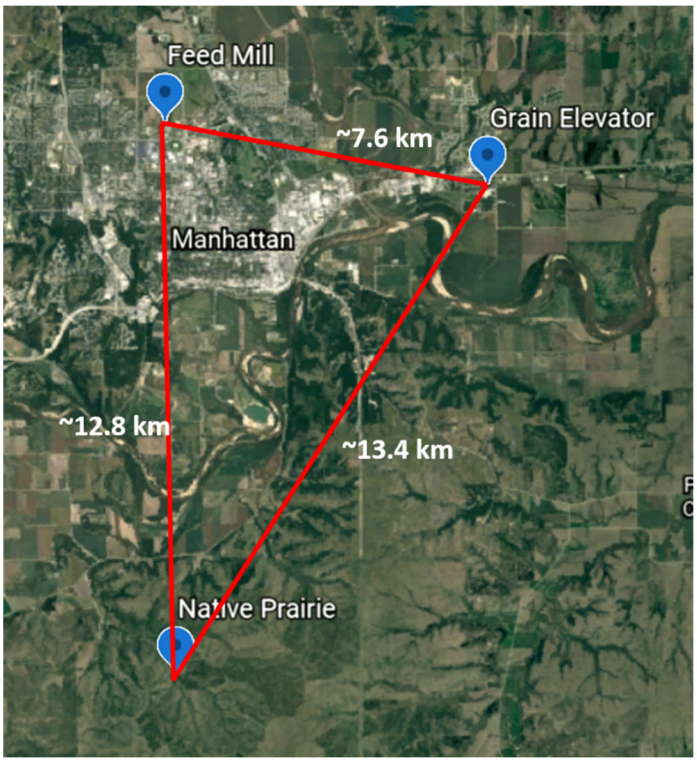

Figure 1. Aerial view of Lindgren trapping locations and approximate distances between locations (km) as estimated by Google Earth (version 9.131.0.1, earth.google.com (accessed on 8 August 2021)).

2.2. Delta Traps

Delta sticky traps (Scentry Delta 1X, Great Lakes IPM Inc., Vestaburg, MI, USA) baited with an aggregation pheromone lure (1:1 ratio of 2 mg Dominicalur-1 and Dominicalure-2, TRÉCÉ Inc., Adair, OK, USA) were deployed prior to the start of Lindgren trapping and between Lindgren trapping periods. This was conducted to determine times to start and stop Lindgren trapping and to provide an additional assessment of seasonal flight activity patterns at each location.

Approximately ~24 h prior to trapping the pheromone lures were opened and placed in an enclosed fume hood to age based on recommendations from previous research [17]. The next day, two Delta sticky traps, each with an aggregation pheromone lure, were positioned at Lindgren trap position 1 and position 6 (Table 1) at approximately 1.5–2 m high. Traps were deployed for 7 d, before collection and processing in the laboratory. Delta traps were deployed once a month when no Lindgren trapping occurred (Table 2). The number of *R. dominica* adults in the traps were counted and recorded for each location. The mean number (±SE) of *R. dominica* adults captured per month at each location were first transformed to $\log_{10}(x + 1)$ scale for statistical analysis [32]. These data were subjected to a two-way analysis of variance (ANOVA) using SAS (SAS Institute, Version 9.4, 2012, Cary, NC, USA) with trapping location and trapping date as the main factors. If the ANOVA was significant ($p < 0.05$) differences among the trapping locations were determined by a Tukey studentized range (HSD) test.

Table 2. Delta and Lindgren trapping dates for 2017 and 2018.

Trial Number	Lindgren Trapping Dates		Delta Trapping Dates	
	2017 *	2018	2017	2018
1	-	1–3 May	-	24 April–1 May
2	-	16–18 May	-	-
3	-	30 May–1 June	-	-
4	-	11–13 June	-	4–11 June
5	-	27–29 June	-	-
6	17–19 July	11–13 July	24–31 July	3–10 July
7	31 July–2 August	24–26 July	-	-
8	16–18 August	8–10 August	21–28 August	31 July–7 August
9	28–30 August	21–23 August	-	-
10	11–13 September	5–7 September	6–11 September	29 August–5 September
11	25–28 September	17–19 September	-	-
12	11–13 October	1–3 October	4–11 October	24 September–1 October
13	23–25 October	15–17 October	-	-

* Trapping study began on 17 July 2017.

2.3. Wheat Preparation

There were six attractants used for testing in the Lindgren traps. Three attractants utilized wheat only, and three attractants used wheat and a pheromone lure. The first type of wheat used for testing was a 13–13.5% m.c. crimped wheat (CW). Prior to testing, 2.2-L aliquots of organic hard red winter wheat were crimped using an antique Tag-Heppenstall moisture meter (C.J. Taglibue MFG. Co., Brooklyn, NY, USA) with two large counter-rotating crimping wheels. It is assumed by crimping the wheat would increase the release of volatiles and potentially improve the chances of *R. dominica* being attracted to the wheat source. The second type of wheat used was an 18–19% m.c. held at 30 °C and 65% r.h. for 8–10 d before testing [33], hereafter termed high moisture wheat (HMW). The increased moisture content held at high temperatures promotes fungi growth on the grain and is thought to increase the moldy volatile cues which could potentially increase attractiveness. The third wheat used was termed pre-conditioned wheat (PCW) [34]. Pre-conditioned wheat consisted of whole-kernel wheat used for laboratory insect colonies at the USDA's—Center for Grain and Animal Health Research Center in Manhattan, Kansas. Lab strain colonies of *R. dominica* were allowed to feed on the wheat kernels for 7–8 weeks, before

being sieved and transferred to new wheat. The previously infested wheat was frozen (−17 °C) for 48 h and sifted to remove any residual *R. dominica* adults before use. The wheat damaged by *R. dominica* feeding was expected to contain the aggregation pheromones in the wheat fines produced by male *R. dominica*, and other chemical cues associated with feeding.

The three wheat types (CW, HMW, PCW) were evaluated with and without the addition of an aggregation pheromone lure (P), 1:1 ratio of 2 mg Dominicalur-1 and Dominicalure-2, (TRÉCÉ Inc., Adair, OK, USA). Thus six attractant combinations evaluated were: crimped wheat (CW); high moisture wheat (HMW); pre-conditioned wheat (PCW); crimped wheat plus pheromone (CWP); high moisture wheat plus pheromone (HMWP); and pre-conditioned wheat plus pheromone (PCWP).

2.4. Lindgren Traps

One week prior to the initial Lindgren trapping, all traps were placed at one of three trapping sites described previously (Table 1). Each testing site had six separate four-funnel Lindgren traps [35] placed approximately 10–12 m apart along a transect. The traps were suspended using electrical wire from a steel rod frame so that the bottom of the collection cup was approximately 0.5–1 m above the ground, according to recommendations from previous trapping studies with Lindgren traps [36]. All vegetation near the Lindgren traps was held under 0.5 m tall throughout the duration of testing.

Approximately 12–24 h prior to insect trapping, all pheromone lures were opened and placed inside the fume hood. On the day of trapping, 50 mL (~100 g) of wheat (CW, HMW, PCW) was placed in the bottom collection cup of the Lindgren trap, either with or without a pheromone lure. The order of the different attractants along the transect was determined using a random number table. One HOBO temperature and light intensity recording logger was attached to the steel crossbar of the Lindgren trap holder at location #1 at each testing site (Onset Computer Corp., Bourne, MA, USA), and set to record the maximum temperature every hour, which was then averaged to a daily high temperature. Lindgren traps were deployed for 48 h. After collection, all wheat samples were sifted, and the number of *R. dominica* adults collected were recorded along with any incidental insects. *Rhyzopertha dominica* were placed inside a 0.5 mL centrifuge tube and held at −80 °C for further genetic testing, which will be reported in a subsequent manuscript. The Lindgren trapping was repeated every two weeks from July–October 2017 and May–October 2018 (Table 2). Trapping concluded each year when Lindgren capture counts were zero at each location, Delta trapping counts reached zero, and temperatures were <13 °C for consecutive days, which is less than the lower threshold for *R. dominica* flight [7].

The number of captures for each attractant type and location were counted and reported for both years. For statistical analysis, *R. dominica* counts were transformed to $\log_{10}(x + 1)$ to satisfy the assumption of normality and homogeneity of variance [32]. In the total number of *R. dominica* adult captured among overlapping trapping periods in Lindgren traps, the differences between each year were determined by a paired *t*-test and the trapping period represented a replicate. Total trap captures were subjected to a three-way ANOVA where trapping year, location, and attractant type were the main effects, and the trapping period represented an individual replicate. Where indicated means (±SE) are reported and subjected to ANOVA using SAS. If the ANOVA was significant ($p < 0.05$), differences were determined by a Tukey studentized range (HSD) test. A Pearson correlation analysis was carried out to determine the relationship between mean temperature and total *R. dominica* captures among all locations and attractants for each individual year and the combined 2017 and 2018 data.

3. Results

3.1. Delta Trapping

In both years there were *R. dominica* adults captured in Delta traps at all locations, with significant differences among locations and trapping periods. In 2017, the main factors

of trap location (F = 20.32; df = 2, 12; p = 0.0011) and trapping period (F = 10.51; df = 3, 12; p = 0.0001) and the interaction of location and trapping period (F = 8.58; df = 1, 12; p = 0.0009) were significant for mean *R. dominica* captures. In 2017, captures were greatest in July at the native prairie location, not significantly different among locations in August and September, and greatest at the feed mill in October (Figure 2A). Combining all trapping periods, the native prairie captured 178 *R. dominica* compared to 95 at the feed mill and 64 at the grain elevator in 2017.

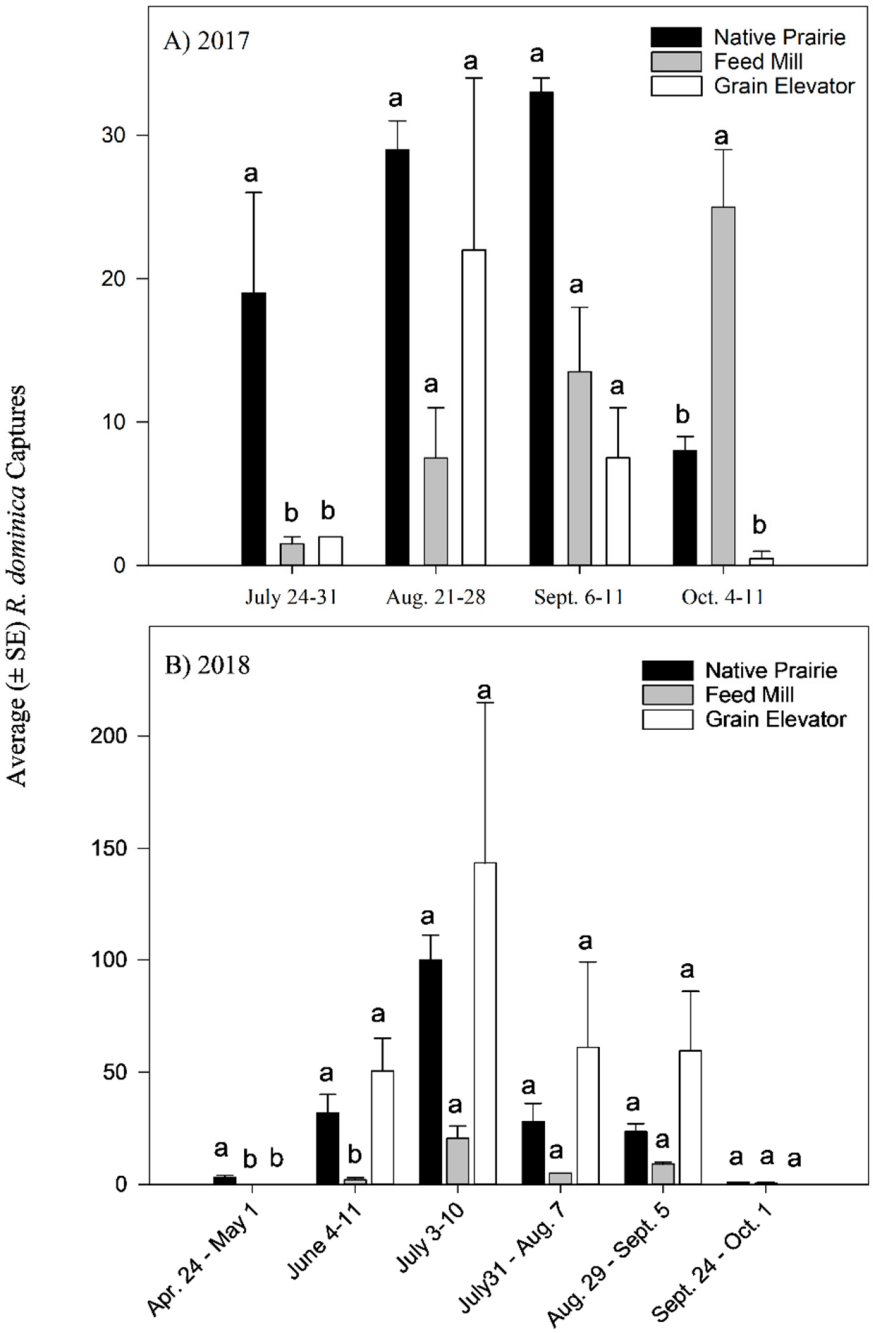

Figure 2. Mean (±SE) *Rhyzopertha dominica* captures in Delta sticky traps during a 7 d trapping period repeated monthly for (**A**) 2017 and (**B**) 2018. The black bars represent the native prairie, gray bars represent the feed mill, and the white bars are the grain elevator. Different letters for each trapping date denote significant differences between the trapping locations (p < 0.05, Tukey studentized range (HSD) test).

In 2018, trapping period ($F = 78.93$; df = 5, 18; $p < 0.0001$), trap location ($F = 39.58$; df = 2, 18; $p < 0.0001$), and their interaction ($F = 4.99$; df = 10, 18; $p = 0.0016$) were all significant. However, significant differences in captures among locations only occurred during the April/May and June trapping periods, where the native prairie and grain elevator had higher counts compared to the feed mill location (Figure 2B). Overall, the grain elevator had more adult *R. dominica* captured in Delta traps, 629, compared to the feed mill and native prairie, 74 and 375, respectively.

3.2. Lindgren Trapping

3.2.1. Seasonal Effect on *Rhyzopertha dominica* Captures

Regardless of trapping location or attractant type, we observed a seasonal trend in total *R. dominica* captures (Figure 3A) for the 2018 trapping season and the combined 2017 + 2018 season. The combined season and 2018, had the highest total *R. dominica* captured during the July trapping period and the lowest captures in May and October (Figure 3A). However, if we combine the trap captures for 2017 and 2018 and average the number of capture for each trapping period, we observe a consistent trapping pattern from late May–early July (Figure 3B). The small dip on trapping period 3, could be due to the less than ideal conditions during the trapping period. During trapping period 3, a rainstorm occurred which could affect the movement of *R. dominica.* After July, we observed a dip in mean *R. dominica* capture and a small uptick in early September, and a final decline through October (Figure 3B). This profile is similar to the mean daily high temperatures at each trapping location (Figure 4A–C). The temperature profiles for each location were similar for 2017 and 2018, except for an increase in temperature during late September 2018.

Additional analysis into the relationship between mean daily high temperatures to *R. dominica* captures was conducted using Pearson correlation. The *R. dominica* captures showed a significant positive correlation between temperature and captures in 2018, but not for 2017 (Table 3). However, if the temperature and capture data is pooled, there was a significant positive correlation indicating an increase in temperature associated with an increase in the number of *R. dominica* captures. The pooled trap capture data, along with 2018, show an increase in total *R. dominica* captures from May through July, which corresponds to increasing daily high temperatures recorded on the HOBO data loggers. As the temperature begins to drop in September and October, the trap captures begin to decrease concurrently.

Table 3. The correlation coefficient for the relationship between mean daily temperature and *Rhyzopertha dominica* captures among all locations and attractant types during 2017, 2018, and the combined years.

Trapping Year	Correlation Coefficient	*p*-Value
2017	0.561	0.1906
2018	0.766	0.0022
2017 and 2018	0.729	0.0003

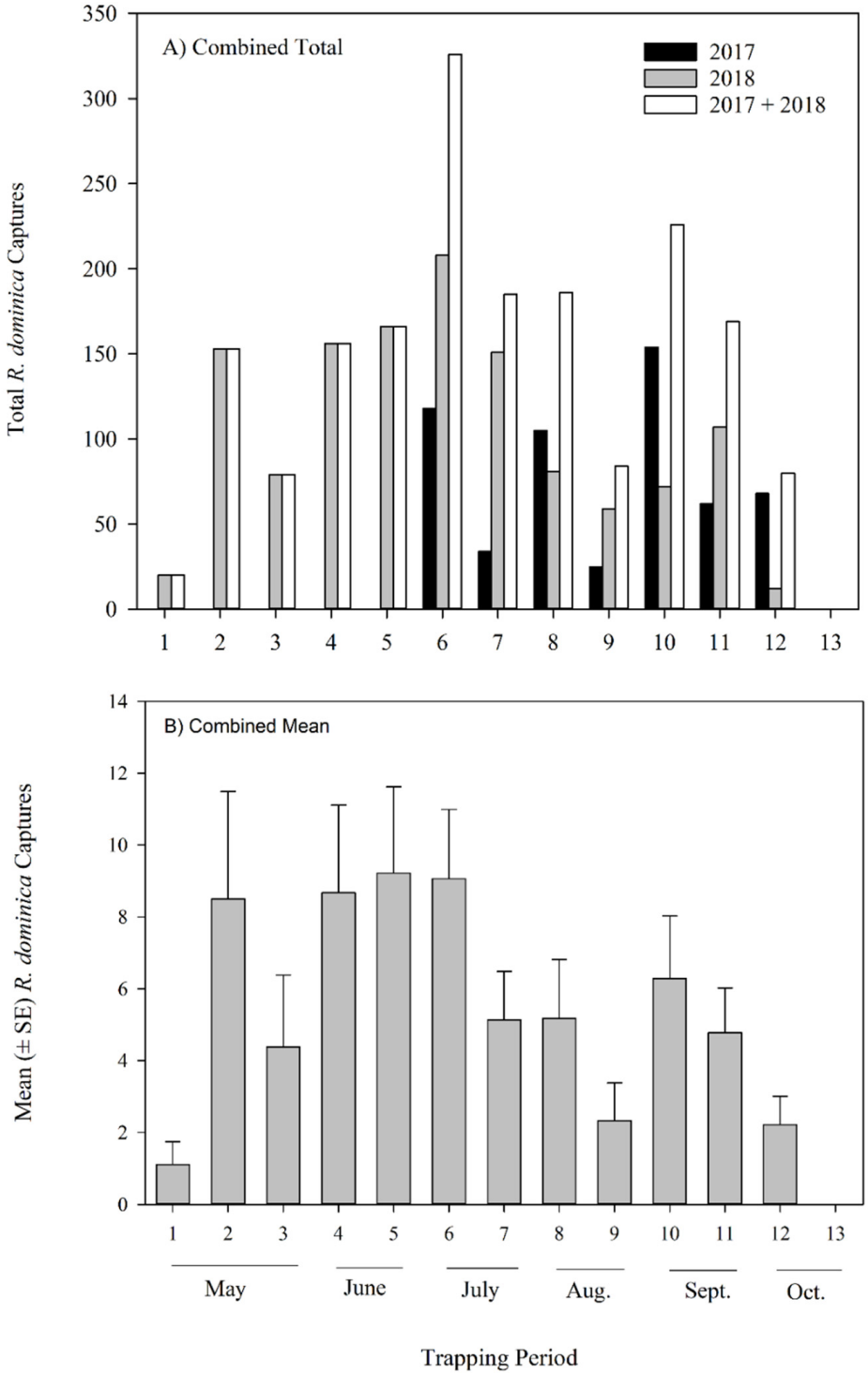

Figure 3. (**A**) Combined total *Rhyzopertha dominica* captures among all locations and attractant types for 2017 (black bars), 2018 (gray bars) and the combined 2017 + 2018 captures (white bars) for each trapping period; (**B**) Combined mean (±SE) *R. dominica* captures among all locations, attractant type, and year of study for each trapping period.

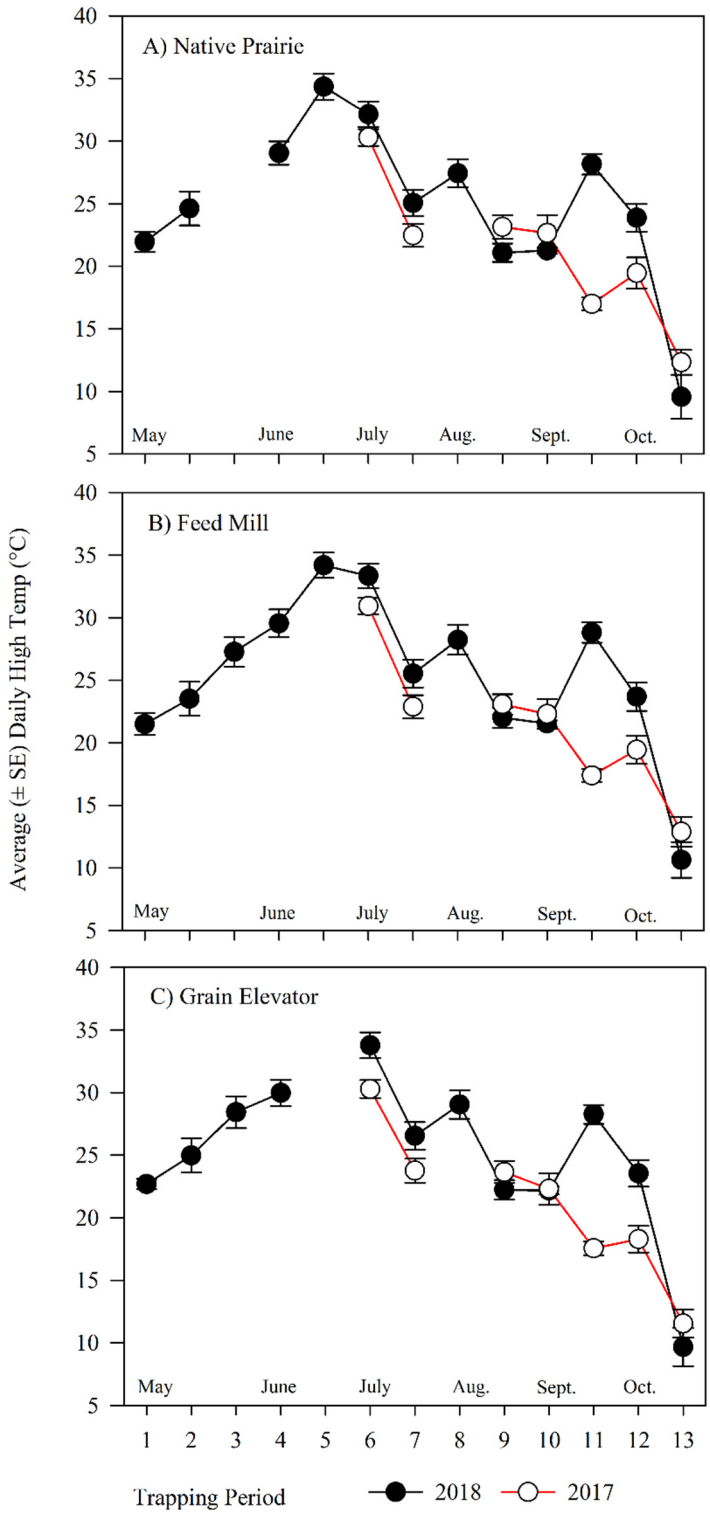

Figure 4. Mean (±SE) daily temperature (°C) for each Lindgren trapping study for (**A**) native prairie, (**B**) feed mill, and (**C**) grain elevator. Red lines and white circles are for 2017 and black lines and black circles are for 2018. If the temperature data file was corrupted, the data was not included in the temperature graphs.

3.2.2. Influence of Ecological Landscape on *Rhyzopertha dominica* Captures

Overall, 1836 adult *R. dominica* were captured over a total of 21 trapping periods during 2017 and 2018. The traps at the feed mill location captured the fewest *R. dominica*

adults, 257, followed by the traps at the native prairie, 275, and grain elevator, 904. In 2017, the total number of *R. dominica* captured ranged from 143–259 among all locations (Figure 5A), with the native prairie location having the most captures. In 2018, the total number captured ranged from 90–761 among all locations (Figure 5A), with the grain elevator having the most captures. The total number captured was greater in 2018, but the number of trapping periods was greater as well. In 2018, there were 13 trapping periods conducted compared to only 8 trapping periods in 2017. If only overlapping trapping periods are compared (trials 6–13; Table 2), the range in *R. dominica* captures was 59–478 among all locations and only the traps at the grain elevator had higher captures in 2018 compared to 2017 (Figure 5B).

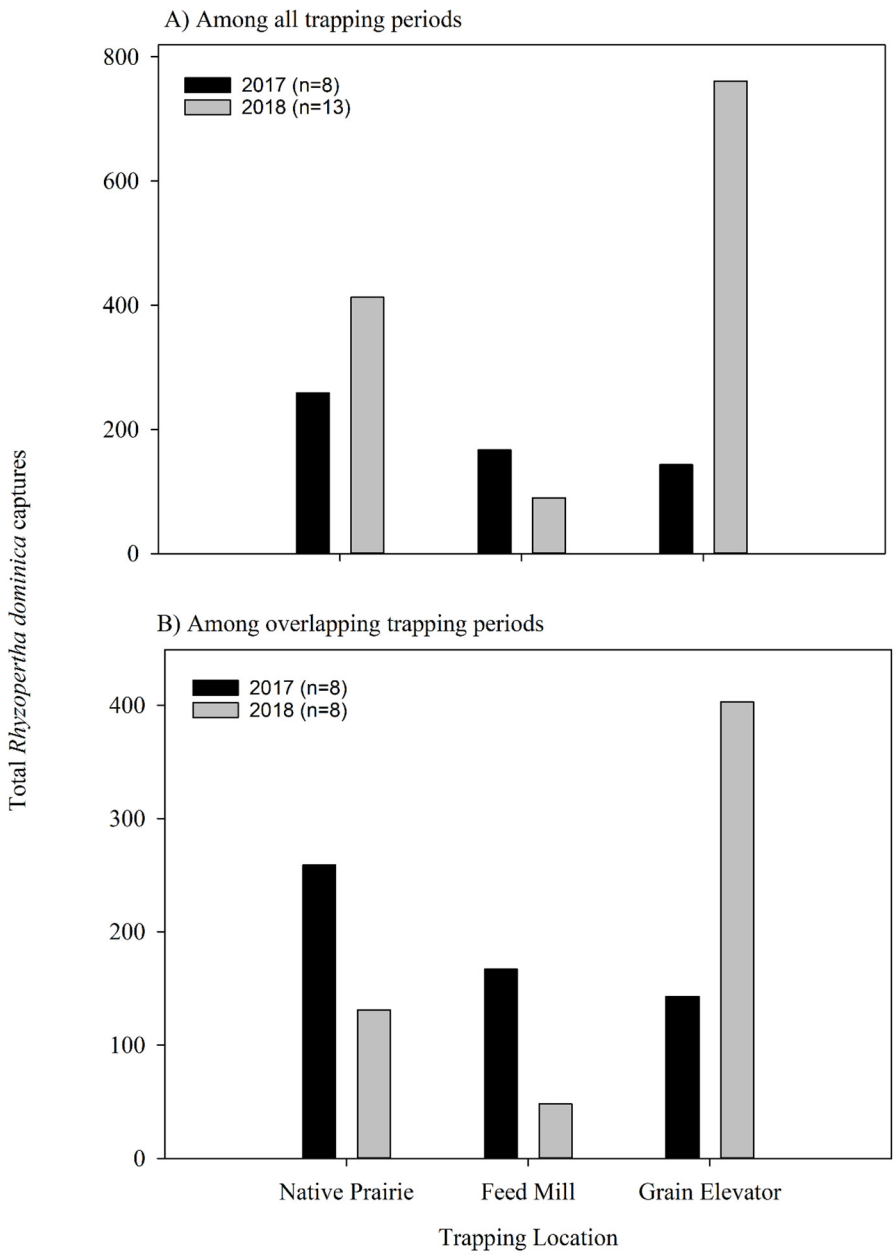

Figure 5. Total *Rhyzopertha dominica* captures in Lindgren traps across all attractant types for (**A**) all trapping periods (2017 trials 6–13 and 2018 trials 1–13) or (**B**) overlapping trapping periods of trials per year (trials 6–13 for 2017 and 2018). Black bars represent 2017 captures and gray bars represent 2018 captures.

Using each trapping period (or trial) as a replicate, we found significant differences in the number of *R. dominica* captures for the main effects of trap location, attractant type, and the interaction of location × year (Table 4).

Table 4. Three-way analysis of mean *Rhyzopertha dominica* captures in Lindgren traps baited with six different attractant types, over two years, at three different locations using each trapping period as a replicate.

Factor	*F*	df	*p*
Trap location	9.44	2, 342	<0.0001
Attractant type	30.72	5, 342	<0.0001
Year	0.94	1, 342	0.33187
Trap location × Attractant type	1.10	10, 342	0.3623
Trap location × Year	9.11	2, 342	0.0001
Attractant type × Year	0.37	5, 342	0.8670
Trap location × Attractant type × Year	1.77	10, 342	0.0647

Since the main effect of trap location was significant, we pooled all the trap captures among each attractant type together for each location (native prairie, grain elevator, and feed mill) and evaluated the *R. dominica* captures for the main effects of trapping location and year of trapping. We excluded the final week of trapping each year because all trap captures were zero, and thus there were $n = 7$ replicates in 2017 and $n = 12$ replicates in 2018 (Table 2 lists trapping dates). The main effect of trapping location was significant ($F = 3.35$; df = 2, 51; $p = 0.0429$) and the interaction of location and year ($F = 4.69$; df = 2, 51; $p = 0.0135$) but the main effect of year was not significant ($F = 0.21$; df = 1, 51; $p = 0.6482$). We further analyzed the effect of trapping location for each individual year (Table 5).

Table 5. Combined total and mean (±SE) *Rhyzopertha dominica* captured in Lindgren traps baited with one of six different attractant types, among three different ecologically different landscapes (each trapping period represented a replicate).

Trapping Location	2017 ($n = 7$)		2018 ($n = 12$)	
	Total Captures	Mean (±SE) Captures [a]	Total Captures	Mean (±SE) Captures [b]
Feed Mill	167	23.9 ± 6.3	90	7.5 ± 2.4 [b]
Native Prairie	259	37.0 ± 13.0	431	34.4 ± 9.1 [a]
Grain Elevator	143	20.4 ± 6.6	761	63.4 ± 10.9 [a]

[a] There were no significant differences among the trapping locations ($F = 0.23$; df = 2, 18; $p = 0.7992$); [b] Different lowercase letters within a column are significant different ($F = 9.40$; df = 2, 33, $p = 0.0006$).

Among all locations, the feed mill was the only location that had lower total and mean captures from 2017 to 2018 (Table 5). In 2018, the native prairie and grain elevator had significantly more *R. dominica* captures compared to the feed mill. Interestingly, the native prairie's mean captures per trapping period were similar for 2017 and 2018, 37 and 34 adults/week respectively. Despite more trapping periods in 2018, the mean captures per week were consistent indicating resident population exists in that location at a somewhat consistent level as indicated by Lindgren trapping. In contrast, the grain elevator had a 3-fold increase in mean captures from 2017 to 2018. A substantial increase in mean captures at this location could indicate a major infestation of the grain being stored which is attracting an increased number of *R. dominica* into that location.

3.2.3. Effect of Attractant Type on *Rhyzopertha dominica* Captures

Previously we determined the main effect of attractant type was significant, therefore (we further analyzed the *R. dominica* captures by pooling the capture data for both trapping years and locations to determine differences between the six attractant types. Again, we

excluded the final week of trapping each year because all trap captures were zero. The mean *R. dominica* captures ranged from 0.3–11.0 among all attractant types (Figure 6). The CWP and HMWP attractants had significantly more *R. dominica* captures compared to attractants containing no pheromone. However, there was no statistical difference between PCW and PCWP. This could indicate that the PCW most likely contains a natural form of the aggregation pheromone, which increased the attractiveness of the wheat compared to CW and HMW. Alternatively, there could be some deterrent/repellent effect in the PCW that is counteracting the synthetic pheromone in the PCWP, which reduced the number of *R. dominica* captures. However, both the PCW and PCWP had significantly more *R. dominica* captures than wheat, CW or HMW, alone.

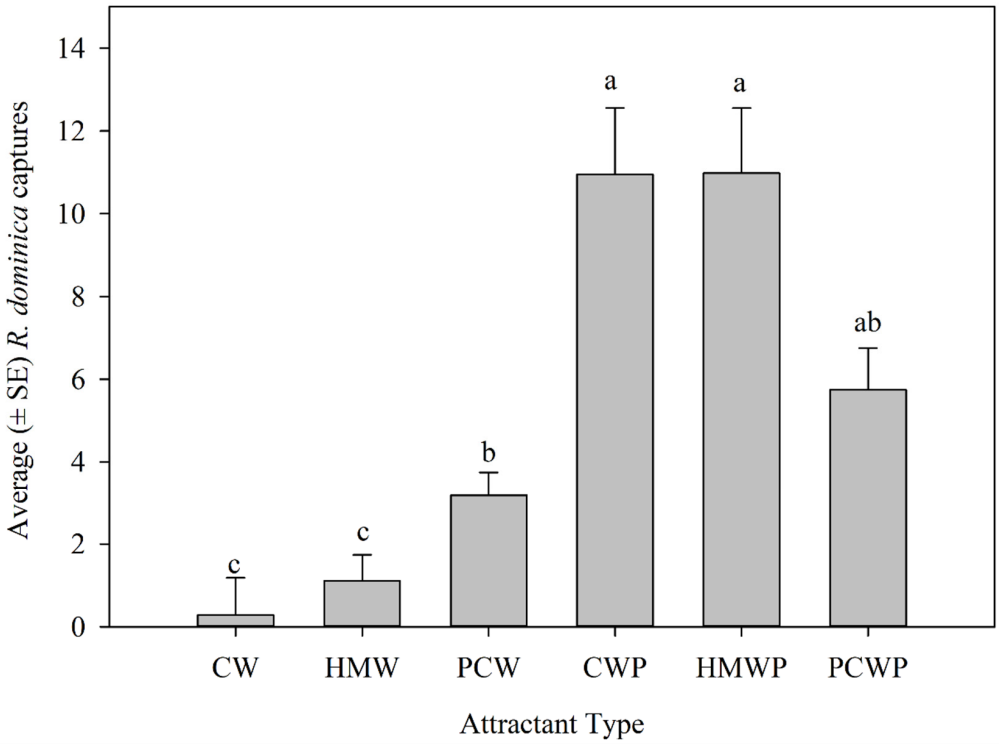

Figure 6. Mean (±SE) *Rhyzopertha dominica* captures over two years and three locations (native prairie, feed mill, and grain elevator) amongst six different attractants (CW: crimped wheat; HMW: high moisture wheat; PCW: preconditioned wheat; CWP: crimped wheat plus pheromone; HMWP: high moisture wheat plus pheromone; PCWP: preconditioned wheat plus pheromone). Different lowercase letters indicant significant differences among each attractant type (F = 32.60; df = 5, 336; $p < 0.0001$, Tukey studentized range (HSD) test).

The mean *R. dominica* captures among all locations for both years, does not tell a complete story. A better depiction of the effect of attractant type can be observed in Figure 7, which illustrates the total trap captures amongst the six attractant types. Similar to mean trap captures, CWP and HMWP had the most trap captures, 624 and 626 respectively. The use of a pheromone had a 39-fold increase in captures between CW and CWP, and a 9.9-fold increase between HMW and HMWP. Similarly, the PCWP had 327 total captures which was a 1.8-fold increase compared to PCW. Furthermore, we examined the impact of pheromone (CWP, HMWP, PCWP) vs. non-pheromone (CW, HMW, PCW) total trap captures among all trapping locations and trapping periods for 2017 and 2018 separately and found there were significantly more *R. dominica* captures in Lindgren traps with pheromones than in traps without pheromones for both 2017 (F = 58.13; df = 1, 142; $p < 0.0001$) and 2018 (F = 52.42; df = 1, 232; $p < 0.0001$). In 2017, the use of pheromones with wheat increased total trap captures by 818%, and in 2018 there was a 543% increase in traps containing a pheromone.

Both the mean and total trap capture data illustrate that the pheromone attractants elicit the strongest response of *R. dominica* consistently among all trapping locations.

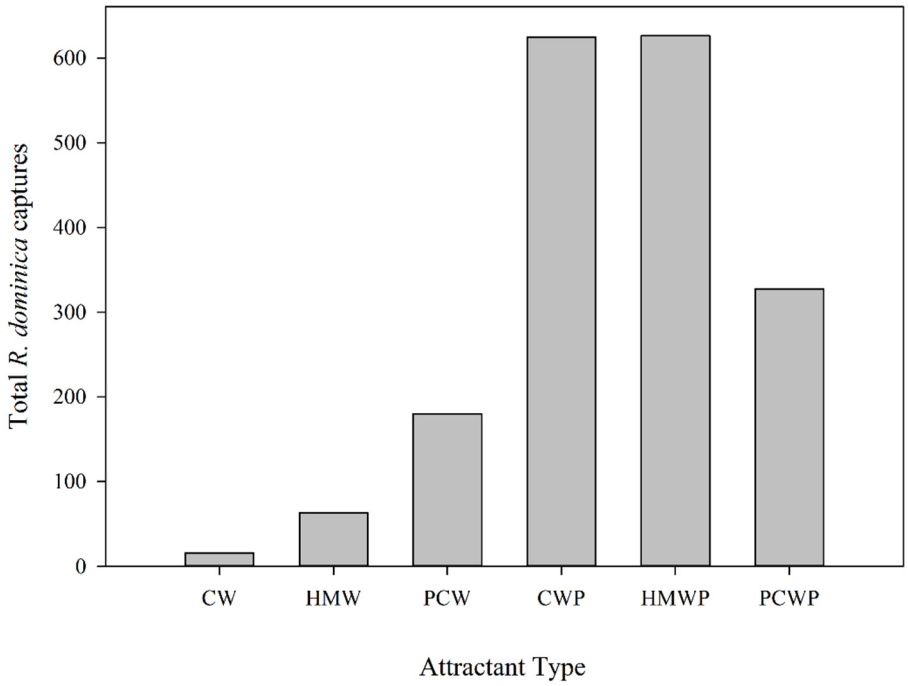

Figure 7. Total *Rhyzopertha dominica* captures over two years and three locations (native prairie, feed mill, and grain elevator) amongst six different attractants. (CW: crimped wheat; HMW: high moisture wheat; PCW: preconditioned wheat; CWP: crimped wheat plus pheromone; HMWP: high moisture wheat plus pheromone; PCWP: preconditioned wheat plus pheromone).

4. Discussion

The results of our study and recent literature reports show that *R. dominica* can be captured consistently in diverse habitats including grain storage and food/feed processing facilities, but also in natural habitats [3,9,20,21,27,29,36]. Though there were fluctuations in traps captures, we consistently captured *R. dominica* from July–October in 2017 and May–October in 2018. This suggests that there are consistent populations of *R. dominica* in the native prairie in Kansas, many kilometers away from grain storage and processing facilities. Previous research has reported that *R. dominica* can reproduce on several different noncereal grain host plants that occur on the native prairie and suggests that there are natural resident populations on the prairie [29]. However, it is not known whether the same population maintains a presence on the prairie or whether a new population of *R. dominica* immigrates into the area each year. The prairie location could be an overwintering location or a stopover point on the way to locating grain being harvested and stored for extended periods of time. It is unknown how *R. dominica* locates stored grain because the adults have not been known to infest the grain in the field in the US, but *R. dominica* can infest the grain soon after it is stored [2]. A previous study reported that *R. dominica* adults were captured in 13 of 34 bins sampled four weeks after newly harvested wheat was stored on 12 Kansas farms [37], which demonstrates an ability to locate and infest newly harvested wheat. It has been proposed that stored product insects are attracted to the grain odor emanating from the eaves and bin openings at the top of the grain mass [37] and orient themselves towards the odor plume. An alternative hypothesis is that a male *R. dominica* could disperse from a non-agricultural habitat and encounter stored grain by chance, begin to feed and release aggregation pheromone to attract other *R. dominica* adults to the stored grain [36] and it is the pheromone that is attracting additional males and females to the stored gran. Therefore, further studies on the population genetics of all *R. dominica* captured at each location is

needed to determine if there are distinct genetic differences between populations collected in agricultural vs. non-agricultural landscapes if the population remains stationary over time and space, or if *R. dominica* is immigrating into new areas weekly, monthly, or yearly.

Similar to previous trapping studies [9], we observed the first captures of *R. dominica* in early spring in 2018 and the last captures during the last week of October for 2017 and 2018. The first captures of *R. dominica* in 2018 occurred at the native prairie location and not near the feed mill or grain elevator, which is similar to previous reports of *R. dominica* seasonal captures [9]. Despite the first year of trapping not starting until July of 2017, we expected to obtain first and last *R. dominica* captures on or about the same time each year because previous research has reported that the climatic conditions do not cause significant differences in the yearly *R. dominica* population activity [9]. The differences in trap capture caused by climate changes would be observed on a short-term or weekly basis compared to yearly activity [31]. The low ambient threshold for *R. dominica* flight initiation is between 16–20 °C and optimum flight conditions are between 31–36 °C [6,7]. Examining the mean daily temps in our study, higher temperatures were correlated with high trap captures, similar to previous *R. dominica* trapping studies [3,9,38]. Further analysis on the impacts of climate on *R. dominica* captures was explored previously [9] but the major conclusions from our study, and the previous study, is that monitoring the temperature on a weekly and monthly basis will allow for predictions to be made based on environmental patterns. Higher daily temperatures will increase *R. dominica* flight activity and render stored grain more vulnerable to infestations compared to cool and windy conditions. In our study, peak flight activity occurred in September 2017 and July 2018, which is consistent with reported peak flight activities reported by others [18,19].

Rhyzopertha dominica will respond to wheat volatiles and pheromone lures independently with variable responses among experiments conducted in the laboratory and/or field assays. Previously *R. dominica* adults have been captured in traps when infested plant materials plus a lure, but there were little or no captures in traps when plant materials alone were used [31]. Additionally, all previous studies [3,9,31,36] have investigated *R. dominica* dispersal and trap captures using Lindgren traps baited with pheromone lures alone. Our study found differences in captures using different types of wheat, suggesting that degraded and previously infested wheat tends to be more attractive, although overall captures were low in the wheat-only treatments. These results shed new light on how *R. dominica* may orient to newly stored grain or locate suitable food sources in non-agricultural landscapes. The odors emanating from the grain, whether that be CW, HMW, or PCW, could be drawing in populations of *R. dominica* from nearby landscapes. The addition of a pheromone, significantly increased the capture rate regardless of which wheat type it was paired with, although interestingly adding pheromone lure to previously infested wheat (PCW) was less attractive than clean wheat or moldy wheat. This interaction needs further evaluation, to determine the mechanism. This information is useful for developing an attract-and-kill or interception strategy for protecting stored grain since attractants that are more attractive than clean newly stored grain might be able to outcompete the odors coming from grain bins. Lindgren traps or other devices could be developed to attract/intercept dispersing insects to a specific location(s) around the stored grain, similar to trap cropping in field agriculture.

Among the wheat-only attractants, the PCW had significantly more captures. In a laboratory bioassay conducted with clean wheat and pre-infested wheat [34], *R. dominica* has been observed to be more attracted to PCW compared to clean wheat. Furthermore, female *R. dominica* walked 35% faster towards the PCW source compared to males [34]. The increase in trap captures in the PCW compared to the CW and HMW in our study were consistent with laboratory trials performed by others [34]. The addition of a pheromone significantly increased trap captures using CW and HMW but there were no significant differences between PCW and PCWP captures. This indicates that PCW reduced the attractiveness of the pheromone. The consistent, but low captures in the CW and HMW, could have been due to the proximity to Lindgren traps containing wheat + pheromone

(CWP, HMWP, PCWP). The traps were placed 10–12 m apart, but the pheromones create an odor plume which may attract adults to the area, and the shape of the Lindgren trap, which gives a silhouette of a tree trunk, could be a visual cue to which the beetles respond [36]. The combination of clean wheat + pheromones in traps appears to provide the best attractant to increase trap capture numbers and can be used for future studies. *Rhyzopertha dominica* mostly likely will not be drawn out of stored grain once they begin feeding, but the use of Lindgren traps around a stored grain elevator could lead to the interception of the adults before they enter the grain storage bin.

In our study, we regularly captured *R. dominica* in pheromone and non-pheromone baited traps in a native tallgrass prairie which was kilometers away from any larger grain storage structures in the area. The *R. dominica* adults captured in the native prairie location could be dispersing individuals that are moving between large grain storage structures and the prairie was serving as a niche habitat and food source [31]. Alternatively, there could be a permanent population of *R. dominica* living on the grasses, seeds, nuts, and other food sources that are naturally found in the prairie. The native prairie landscape location in this study contained such wooded and grassland areas similar to those described in previous studies [26] and thus could be potential hosts for *R. dominica*. Additional studies are underway examining the population dynamics of the *R. dominica* captured in this study, along with subsequent trappings in 2018 and 2019. The genetic attributes of this future study will allow us to better understand the population structure of *R. dominica* captured in the prairie and how it compares to those captured near the grain elevator and feed locations and potentially determine if the *R. dominica* found in the prairie are in transit towards grain storage or other grain sources such as the mill, if the same population exists year to year, or if a new genetically different population immigrates into grain storage sites each year.

5. Conclusions

As the temperatures increase from spring to summer, the stored grain will most likely be under greater insect pressure due to the increased flight activity of *R. dominica* and increased volatiles produced during grain harvest, transportation, and storage. Understanding how temperature impacts *R. dominica* captures both near grain storage elevators and natural prairie lands, are key for employing integrated pest management (IPM) tactics to protect stored grains. As the outdoor environmental temperatures begin to rise, especially over the lower threshold limit, more attention must be used to observe changes insect trap captures because of the potential increase in insect immigration into a facility or stored grain due to the increase in insect movement. In addition, understanding when peak flight occurs can help pest management professionals understand when additional IPM treatment options are needed. Actively monitoring for infestations in grain bins or insect traps is one tool to identify when populations levels are changing. In this study, we found seasonal captures of *R. dominica* can be monitored by Delta sticky traps and Lindgren funnel traps among three ecologically diverse landscapes. The use of a pheromone lure in combination with wheat significantly increased trap captures in the Lindgren funnel traps compared to traps without a pheromone. Increasing the moisture content and previously infested grain did not improve trap captures compared to crimped wheat. Therefore, we propose that the use of crimped wheat plus a pheromone would be the easiest and most effective Lindgren attractant to monitor *R. dominica* seasonal patterns. However, instead of Lindgren trap availability, the use of Delta sticky traps would be a valuable and dependable alternative.

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