The role of predispersal seed predators and their parasitoids for *Ruellia humilis* reproduction

Authors: Julia Clark, Dr. Janette Steets*
Department of Plant Biology, Ecology, and Evolution, Oklahoma State University

Abstract

Plant reproductive success is greatly affected by predispersal seed predators and their parasitoids. *Ruellia humilis* is an herbaceous plant with a dimorphic flowering system in which individual plants can produce both open showy flowers (chasmogamous, CH) and closed flowers (cleistogamous, CL) that can only self-fertilize. The objectives of this study were to (1) determine the extent of among population variation in *R. humilis*-seed predator-parasitoid interactions and (2) determine whether seed predation and parasitoid rates vary between CL and CH fruits of *R. humilis*. We found considerable among population variation in seed predation and parasitism rate. Additionally, we found that the seed predator, *Tripudia rectangula*, preferentially attacked CH fruits. In contrast, the wasp parasitoids of the seed predator preferentially attacked seed predators in CL fruits. Our findings indicate that while parasitoids have been found to influence seed predation rates by the seed predator, the parasitoids themselves do not drastically affect the reproductive success of *R. humilis*. Given the preference of the seed predators for CH fruits, this ecological interaction may be a potent selective force for reproductive traits of *R. humilis*.

Keywords: *Chasmogamous*, *Cleistogamous*, Parasitoid, Seed Predator

Introduction

Pre-dispersal seed predators, which consume seeds prior to their dispersal from the maternal plant, can have significant ecological and evolutionary consequences for plant populations. To be able to predict longer terms ecological and evolutionary consequences of plant-seed predator interaction, it is critical to understand the extent of spatial and temporal variation in the interaction (Kolb *et al*. 2007).

Seed predation rates are influenced by both the abiotic and biotic dimension. From the biotic perspective, variation in the presence of natural enemies, such as parasitoids that attack the seed predator, may cause spatial and temporal variation in rates of seed predation (Kolb *et al.* 2007). Thus, it is relevant to study the effect parasitoids of the seed predator have on the population and determine whether their presence causes any major changes in seed predation rates.

While looking at plant populations, it is important to consider that variation in seed predation may also be due to variation among plants in their traits, such as mating system. Some plants have an obligate mixed mating system by producing open showy flowers (chasmogamous, CH) that can outcross or self-fertilize and closed flowers (cleistogamous, CL) that can only self-fertilize. Munguia-Rosas *et al.* (2013) found that seed predation by a noctuid moth was higher for CH fruits than for selfed CL fruits. In this study, researchers looked at how both seed predators and parisitoids overall affected the seeds attacked. This helped determine which seeds were surviving in the spatial variation included in (Table 1), so that the rates of the CL vs CH fruit seeds, that were not being eaten or were being eaten, could be tracked and compared in relation to the genetic pool.

Ruellia humilis is an herbaceous plant commonly known as wild petunia. *R. humilis* has been recorded in Midwestern states such as Kansas, Texas, and Ohio (Long 1962) where the active seed predator *Tripudia rectangula* is present (Pogue 2009). In its larval form, *T. rectangula* enters the fruits of *R. humilis* and consume the seeds (Pogue, 2009). *R. humilis* is known to produce both CL and CH flowers on a single plant (Long 1961). *R. humilis* begins to produces CH flowers at the end of spring, whereas CL flower production commences mid-summer (Long 1961). A plant may reproduce via both flower types through the fall (Long 1961).

The objectives of this study were to (1) determine the extent of among population variation in *R. humilis*-seed predator-parasitoid interactions and

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Table 1 *- Latitude, longitude, and elevation for 13 sampled R. humilis populations*

Population	Site	State	Latitude (N)	Longitude (W)	Elevation (ft)
P1	OSU Cross Country Fields	OK	36.13	97.07365	977
P2	Lake Carl Blackwell	OK	36.12368	97.210367	991
P3	Osage Hills State Park	OK	36.75713333	96.17565	960
P4	Ninnescah Reserve	KS	37.5338667	97.676883	1323
P5	Gerber Reserve	KS	37.67765	97.946517	1443
P6	Sand Hills State Park	KS	38.1296	97.8366	1504
P7	Sanborn Lake	OK	36.1547333	97.07605	952
P8	Coughlan Reserve	KS	38.3170833	96.336417	1293
P9	Ross Reserve	KS	38.4943667	96.336317	1270
P10	Tallgrass Prairie Preserve 1	OK	36.752033	96.363417	1000
P11	Tallgrass Prairie Preserve 2	OK	36.7991833	96.413033	1105
P12	Tallgrass Prairie Preserve 3	OK	36.8402833	96.473667	1139
P13	Lake Pawhuska	OK	36.6442833	96.390567	945

(2) determine whether seed predation and parasitoid rates vary between CL and CH fruits of *R. humilis*.

Materials and Methods

In July and September 2015, CL and CH fruits were randomly collected from up to 25 individuals in each of 13 *R. humilis* population in OK and KS (Figure 1, Table 1). Fruits were stored in glassine envelopes until dissection in the laboratory. Fruits were individually dissected and examined under a Leica S8APO microscope. For each fruit, we recorded fruit type (ex: CL vs. CH), number of intact seeds, number of partially eaten seeds, number of aborted seeds, *T. rectangula* presence and evidence (e.g, hole in fruit wall, presence of caterpillar or adult moth, frass), and braconid wasp parasitoid presence and evidence (e.g., presence of wasp larvae or adults). Any extra notes or observations were also recorded at this time. Fruits that were not ripe or that were picked too early to evaluate were discarded from data collection.

We used Pearson correlation analysis to determine the strength of the relationship between seed predation and parasitoid attack rates among populations. We performed chi-square analysis to determine whether seed predators differentially attack CL vs. CH fruits and to determine whether braconid wasp parasitoids preferentially attack seed predators within CL vs. CH fruits. All analyses were performed in SPSS.

Results

Across populations, there is considerable variation is seed predation rates, with attack rates

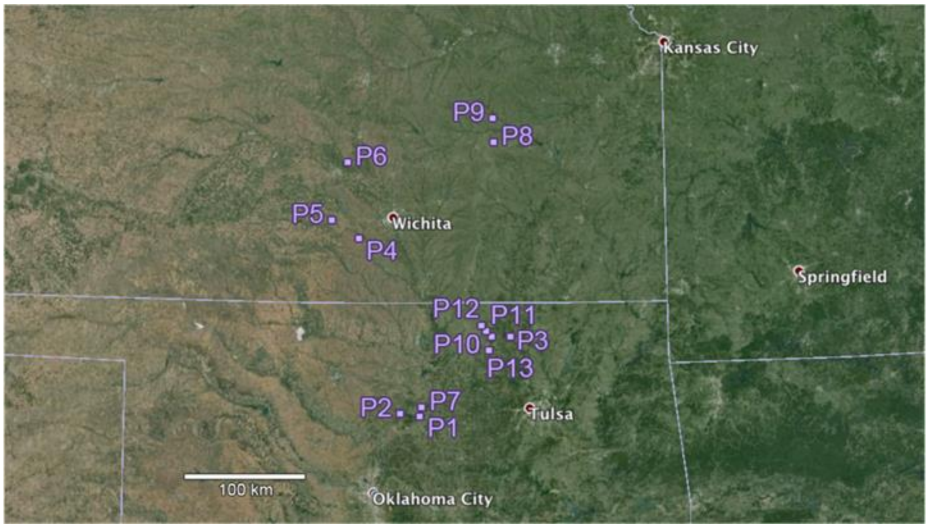

Figure 1 *- Map of 13 R. humilis populations sampled in 2015.*

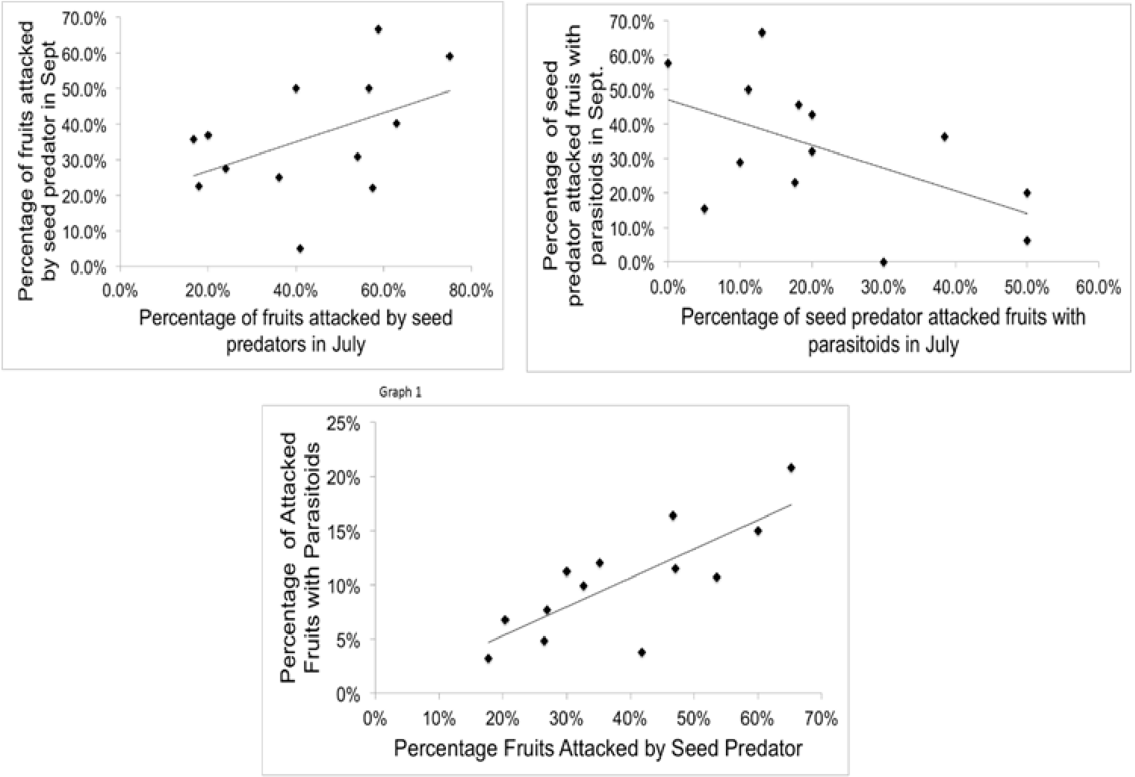

Figure 2 *- Among-population variation in (A Top Left) seed predator attack rates in July and September 2015 and (B, Top Right) braconid wasp parasitoid attack rates in July and September 2015. (C, Bottom Center) Among-population relationship between parasitoid and seed predator attack rates, pooled across the 2015 season.*

ranging from 5-70% of collected fruits attacked within a population (Figure 2A). Populations with high seed predator attack rates in July tended to also experience high seed predator attack rates in September (r = 0.5, *P* = 0.1; Figure 2A). Parasitoid attack rates also varied spatially, with populations experiencing no braconid wasp parasitism to up to 67% of fruits with seed predators being attacked by parasitoids on a given sampling date (Figure 2B). Populations with high parasitoid activity in July experienced low parasitoid activity in September (r = -0.54, *P* = 0.05; Figure 2B). Pooled across the season, there is a positive correlation between seed predator

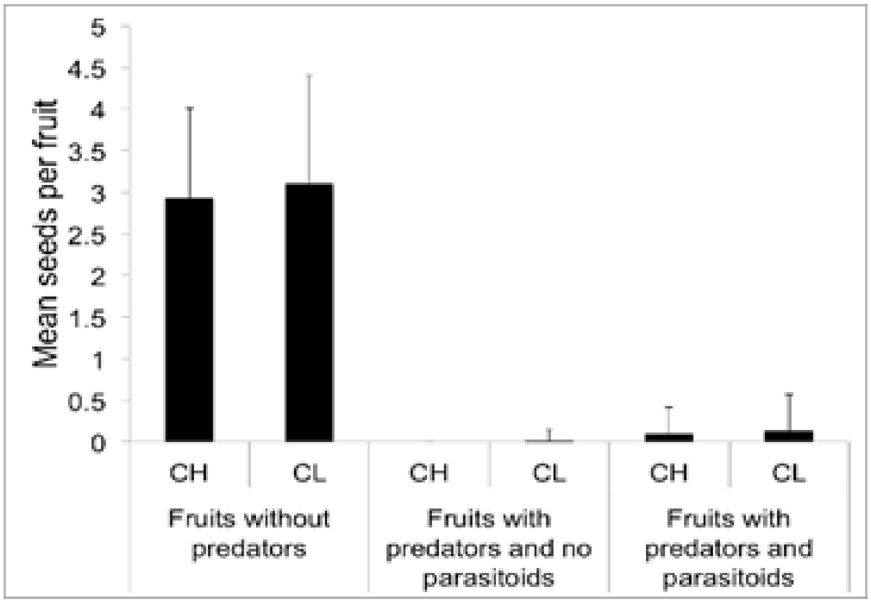

Figure 3 *- Mean seed production per CL and CH fruits in the absence of seed predators, the presence of seed predators and not parasitoids, and the presence of seed predators and parasitoids.*

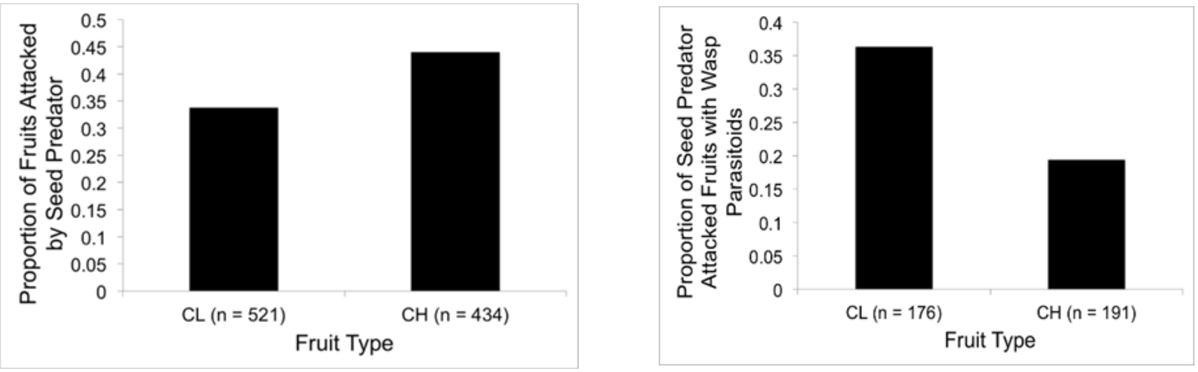

Figure 4 *- The preference of seed predators (A) and their braconid wasp parasitoids (B) for CL vs CH fruits.*

attack rate and parasitoid activity (r = 0.78, *P* = 0.002; Figure 2C).

Overall seed production in CH fruits vs CL fruits does not differ, with an average of three seeds per fruit (F1, 591 = 0.5, *P* = 0.47; Figure 3). Seed predators significantly reduce the number of seeds per fruit regardless of seed type, typically consuming all seeds within a fruit (F1, 591 = 478, *P* < .0001; Figure 3). Across populations, parasitoids rescue 0.1 CH seeds and 0.14 CL seeds per fruit from being consumed by the seed predator (Figure 3).

Seeds predators attack CH fruits proportionally more than CL fruits (X^2 = 10.5, *P* = 0.001; Figure 4). Wasp parasitoids of seeds predators are more frequent in CL fruits than CH fruits (X^2 = 13.3, *P* = 0.0003; Figure 4).

Conclusion

Overall, we found considerable variation in seed predation rates across populations. Interactions between seed predators and their parasitoids influence seed predation rates of *R. humilis*. In a congener, *R. nudiflora*, parasitoids were also found to significantly influence seed predation rates (Abdala-Roberts *et al.* 2009). Given the high rates of seed predation found in many populations coupled with the strong fitness effects of the seed predator, it is likely that *T. rectangula* serves as a potent evolutionary force in *R. humilis* populations.

We found higher rates of seed predation of CH fruits. This differential predation of CH vs. CL fruits may shape mating system evolution of *R. humilis*. In particular, the differential attack rate may result in an evolutionary shift towards a CL dominated plant pool to avoid getting attacked by the seed predators as CH fruits are the first to suffer by attack (Munguia-Rosas 2015). Interestingly, wasp parasitoids attack caterpillars in CL fruits at a higher rate than those in CH fruits, but that may have been due to the availability of CL fruits in higher numbers later on in the summer.

Differential predation of fruit types may also affect population genetic variation, as the CL fruits do not have the genetic diversity of the CH fruits. More work is need to determine the overall germination rate of CL seeds vs CH seeds as well as their survivorship, to determine the demographic and population genetic consequences of differential seed predation. No work has currently been done on the survivorship of *R. humilis* CH vs. CL seeds or investigation into biodiversity and inbreeding depression later on in this species' life. Due to the suggested success of the CL in *R. humilis*, it is important to later find whether or not the seeds go on to outperform in germination and survivorship against the CH seeds.

Acknowledgements

This research was supported by a scholarship granted to freshman researchers via the Oklahoma State University Howard Hughes Medical Institute (HHMI) program.

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