



## Spatial Variation in the Strength of a Trophic Cascade Involving *Ruellia nudiflora* (Acanthaceae), an Insect Seed Predator and Associated Parasitoid Fauna in Mexico

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### ABSTRACT

Spatial variation in the strength of herbivore top-down control represents an important source of variation in plant fitness measures and community structure and function. By measuring seed predator (larvae of a Noctuid moth) and parasitoid impacts on *Ruellia nudiflora* across a broad spatial scale in Yucatan (Mexico), this study addressed the following: (1) to what extent does seed predator and parasitoid attack intensity associated with *R. nudiflora* vary spatially? (2) Does parasitoid attack result in a positive indirect effect on the plant, and does the intensity of this effect vary spatially? During the peak of fruit production (late June–early July) of 2005, we collected fruits from 21 *R. nudiflora* populations and grouped them into four regions: center, east, north and south. For each fruit we recorded: observed seed number, number of seeds eaten, seed predator presence, parasitoid presence and number of seeds ‘saved’ by parasitoids. Seed predators attacked ca 30 percent of fruits/plant on average, while parasitoids were found in 24 percent of seed predator-attacked fruits. Results indicated spatial variation in seed predator and parasitoid attack levels; interestingly, a contrasting spatial gradient of attack intensity was observed: populations/regions with greatest parasitoid attack levels usually had the lowest seed predator attack levels and vice versa, suggesting top-down control of parasitoids on seed predators. We observed a weak overall indirect impact of parasitoids on *R. nudiflora* (4% seeds ‘saved’ on average), which nonetheless varied strongly across populations (e.g., close to 14% seeds saved at one population). Findings indicate a geographical structuring of interaction strengths across populations, as well as spatial variation in the strength of parasitoid cascading effects on plant reproduction.

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*Key words:* density dependence; seed predation; top-down effects; tritrophic; Yucatan.

SPATIAL VARIATION IN THE INTENSITY of top-down and bottom-up effects represents an important source of variation in plant and herbivore fitness measures, population dynamics and community properties (Aquilino *et al.* 2005, Denno *et al.* 2005, Gripenberg & Roslin 2007). Likewise, understanding which factors drive spatial differences and heterogeneity in multitrophic interaction strengths is of fundamental importance in community ecology (Hunter & Price 1992, Gripenberg & Roslin 2007). Despite this, tritrophic studies and food web experiments have usually not considered spatial variation in direct and indirect effects or even been conducted at large spatial (*i.e.*, regional) and temporal (*i.e.*, multi-year) scales (Levin 1992, Dyer 2007). For example, trophic cascades, defined as ‘an increase in plant primary productivity due to negative effects of predators on herbivore biomass’ ( Hairston *et al.* 1960, Paine 1980, Power 1990, Polis *et al.* 2000), have been proposed as an important indirect effect influencing the structure and function of communities (Holt 2000, Polis *et al.* 2000, Dyer 2007). Nonetheless, the strength and occurrence of trophic cascades has not been properly tested either temporally or spatially (Dyer 2007;

but see Barton 1986, Sinclair *et al.* 2000), limiting our understanding on the ubiquity of this type of effect.

Furthermore, very few studies on trophic cascades in natural systems have looked at the positive impact of parasitoids on plants (Gómez & Zamora 1994), despite the importance these carnivores have for insect herbivore control (Hawkins *et al.* 1997). In addition, parasitoid species or guilds may vary in their efficiency to mitigate herbivore impacts on plants and such differences may determine the overall strength of the trophic cascade. Only a handful of studies have looked at spatial variation in the intensity of herbivore and parasitoid attack levels but these studies did not evaluate the impact of parasitoids on producers (Peary Salomon & McNaughton 1979, Kruess 2003, Thies *et al.* 2003, von Zeipel *et al.* 2006).

A number of studies have shown that plant seed production and recruitment, both at the population and at the community levels, may in some cases be strongly impacted by insect predispersal seed predators (Leimu & Lehtilä 2006, Maron & Crone 2006). In turn, insect seed predator attack rates frequently vary both spatially and temporally (Kolb *et al.* 2007) due to abiotic conditions (Solbreck 1991, Roy & Thomas 2003) and/or parasitoid attack rates (Gómez & Zamora 1994, van Loon *et al.* 2000, von Zeipel *et al.* 2006). Such variation in the intensity of herbivore and parasitoid

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impacts may result in a geographic structuring of plant–herbivore–parasitoid interactions resulting in different ecological and evolutionary outcomes at each site (Thompson 2005). Thus, a tritrophic approach that considers spatial variation in third trophic level attack intensity may help explain spatial variation in insect seed predator attack levels and plant fitness measures and productivity, and have potentially important implications for the evolution of the plant–herbivore antagonism across sites (*e.g.*, strength of herbivore selection on plant traits and coevolutionary processes; Thompson 2005).

The goal of the present study was to quantify the variation in strength of effects of a predispersal seed predator and its associated parasitoids on the herb *Ruellia nudiflora* Engelm. and Gray Urb. (Acanthaceae) using a multipopulation approach across a broad spatial scale in Yucatan (SE Mexico). Specifically, we asked the following: (1) to what extent does seed predator and parasitoid attack intensity associated with *R. nudiflora* vary spatially? (2) Do parasitoids have a positive indirect effect on the plant, and assuming they do, is there spatial variation in the strength of this trophic cascade that helps explain variation in seed predator attack rates? Results are discussed in the context of potentially important biotic and abiotic factors influencing the observed patterns.

## METHODS

**STUDY SPECIES.**—*Ruellia nudiflora* is a self-compatible perennial herb, which typically measures 20–30 cm in height, and is distributed from Texas to SE Mexico. It grows mostly in disturbed open areas (Tripp 2007) and has a wide distribution across the state of Yucatan (Mexico). It has a mixed reproductive system as it produces both open flowers (chasmogamous = CH) and flowers that remain closed (cleistogamous = CL) and self-pollinate obligately. The peak of CH flower production occurs from mid June to late July. Flowers are visited by at least six bee species (*e.g.*, *Apis mellifera*, *Trigona fulviventris*) and five butterfly species (*e.g.*, *Microtia elva*). *Ruellia nudiflora* is assumed not to be pollen-limited because CH flowers self-pollinate automatically during corolla dehiscence, as anthers come into contact with the stigma when the corolla falls off the plant (Abdala-Roberts *et al.* 2009). Fruits are dry and dehiscent, each one normally bearing 10–12 seeds. Seeds remain viable for 3–4 mo, after which the germination rate decreases considerably (C. Cervera-Herrera, unpubl. data), suggesting that this species

does not have a seed bank (nor seed dormancy). Finally, *R. nudiflora* has limited seed dispersal, as most seeds fall within a 2 m radius around the parent plant.

Fruits are attacked by larvae of an unidentified species of moth (Lepidoptera: Noctuidae), which feed on seeds before their dispersal and are the only recorded seed predator of *R. nudiflora* in the Yucatan Peninsula (V. Parra-Tabla, unpubl. data). Adult female moths oviposit on recently formed fruits, and the larva grows inside the developing fruit and consumes all the seeds. Before pupating, each larva excavates a tunnel that the adult uses to exit the fruit (L. Salinas-Peba, pers. obs.). Typically, only one larva is found per fruit. Preliminary data indicate that attack intensity does not vary between fruits of CH and CL flowers (P. Carbajal-Borges, unpubl. data). Finally, recent work suggests that herbivore larvae are attacked by up to seven species of parasitic wasps, some of which belong to Braconidae (*e.g.*, *Bracon* sp., *Chelonus* sp.) and Chalcididae (M. Cuautle-Arenas, unpubl. data).

**STUDY AREA AND SAMPLING.**—From late June 2005 to early July 2005 (peak of fruit production), a total of 21 *R. nudiflora* populations located in the state of Yucatan (SE Mexico) were visited. Populations were grouped into four regions based on their location within the state: center (20°48'12" N, 89°36'17" W; five populations), east (20°44'53" N, 88°7'52" W; five), north (21°9'53" N, 89°31'34" W; six) and south (20°1'36" N, 89°14'50" W; five; see Fig. S1). These regions differed strongly in both abiotic (temperature and rainfall) and biotic (type of forest) characteristics (see Table 1) despite geographical proximity in some cases. Distances between populations within each region ranged from 1.3 to 60.9 km (Fig. S1), and all sampled populations were found at disturbed open areas next to tropical dry forest fragments (Table 1); within each region, biotic and abiotic conditions across populations were similar (*e.g.*, soil depth and moisture, vegetation structure). Populations all had similar plant densities, although their size varied from 40 to > 100 flowering and nonflowering *R. nudiflora* individuals. Each population was divided into four to five blocks (3 × 4 m each; separated by 3–5 m) to control for within-population variation in the response variables measured; each block contained three to ten plants. Statistical analyses were conducted both at the regional and population level to gain some insight on the relevant spatial scale at which to measure the strength of second and third trophic level effects on *R. nudiflora*. Reproductive phenology

TABLE 1. Summary of relevant abiotic and biotic features of *Ruellia nudiflora* study regions. Temperature and precipitation data from Flores and Espejel (1994), while seed predator and parasitoid data are from this study. PFSP, percent of fruits attacked by the seed predator; PFP, percent of attacked fruits with parasitoids.

Region	Forest type	Temperature (annual mean °C)	Rainfall (mean mm/yr)	Populations sampled	PFSP (mean ± SE)	PFP (mean ± SE)
North	Thorny deciduous tropical forest	25.4	500	6	45.1 ± 2.5%	9.0 ± 1.8%
Center	Deciduous tropical forest	26	940	5	29.1 ± 2.4%	29.7 ± 4%
South	Semi-evergreen tropical forest	25.9	1200	5	26.0 ± 2.4%	24.0 ± 4.1%
East	Semi-evergreen tropical forest	25.3	1150	5	15.0 ± 1.7%	44.0 ± 4.8%

for *R. nudiflora* in the south and east regions is usually 1 or 2 wk ahead compared with the north and center, and this is mostly due to the frequency and intensity of initial rains in May and June (both greater in the southeast). Thus, we first sampled east and south populations during the last week of June, while the first week of July we sampled populations from the center and north. In this way, we were able to collect fruits during the peak of fruit production of each region, as well as minimize differences in seed predator and parasitoid attack levels due to differences in sampling time relative to fruiting phenology.

An average of  $30 \pm 9$  plants were chosen per population and matched in terms of leaf number, height and display size. Depending on their availability, 6–12 completely mature fruits (dry, brown) were collected from each plant. Collecting only mature fruits was important to avoid reducing the window of time for parasitoid arrival. Fruits were placed in paper bags and transported to the laboratory where they were dried and opened ( $N = 4061$ ); before the fruits were opened, herbivores finished eating all seeds within fruits in most cases. For each fruit the following were recorded: observed (counted) number of seeds, number of seeds eaten (number of missing seeds based on the funicles that connect each seed with the placenta), seed predator presence (direct [larva] or indirect evidence [frass]), parasitoid presence (direct [adult wasp or larva] or indirect [empty cocoon or mummy] evidence) and number of seeds 'saved' by parasitoids. The number of saved seeds was calculated only for plants that were attacked by the seed predator for which we counted remaining intact seeds in fruits that had evidence of both predator larvae and a parasitoid presence; such seeds were not consumed by the seed predator because the parasitoid killed it before it could do so. Unparasitized herbivore larvae typically consume all seeds within a fruit. Total (potential) number of seeds/fruit was equal to the observed number of seeds plus the number of seeds eaten. The response variables analyzed were then: proportion of fruits attacked by the seed predator per plant (attacked/total number of fruits per plant), proportion of seed predator-attacked fruits with parasitoid per plant (number of fruits with parasitoid/number of fruits with seed predator per attacked plant) and the proportion of saved seeds per plant (number of remaining seeds in fruits with both organisms/total number of seeds per attacked plant). The proportion of seeds saved is a direct measure of the cascading effect of parasitoids on *R. nudiflora* seed production.

**STATISTICAL ANALYSES.**—The data were analyzed using generalized linear mixed models by means of the GLIMMIX macro in SAS ver. 8e (SAS Institute Inc. 2002). First, we evaluated the effects of region (fixed), population (random, nested within region) and block (random, nested within population) on the proportion of fruits attacked by seed predator larvae per plant to test for spatial variation in seed predator attack levels. The number of fruits/plant was used as a covariate to account for potential differences in fruit display size per plant, which could be related to seed predator attack intensity (the covariate showed previously not to interact with the region effect). This model assumed a binomial distribution and logit link function. We also used linear and quadratic regression models to explore the relationship between population size (log-transformed)

and proportion of attacked fruits by the seed predator ( $N = 21$  in both cases).

The GLIMMIX macro in SAS was also used to evaluate the effects of region (fixed), population (random, nested within region) and block (random, nested within population) on the proportion of seed predator-attacked fruits with parasitoids and the proportion of seeds saved by parasitoids in order to test for spatial variation in parasitoid attack levels and in their net effect on the plant, respectively. The proportion of seed predator-attacked fruits per plant was used as a covariate for the analysis of proportion of fruits with parasitoid to control for differences in herbivore abundance per plant which could influence parasitoid attack intensity (the covariate did not interact significantly with the region effect); both models assumed a binomial distribution, used a logit link function and only considered plants that had been attacked by the seed predator (*i.e.*, those with at least one fruit attacked, representing *ca* 65% of all experimental plants). Finally, we used linear and quadratic regression models to explore the relationship between number of seed predator-attacked plants (log-transformed) and the proportion of fruits with parasitoids (square root-transformed to achieve normality) ( $N = 21$  in both cases).

Data for all analyses using GLIMMIX showed acceptable extra-dispersion scale values ( $< 3$  in most cases). The LSMEANS statement was included to test for differences between main effect levels when the region effect was significant (Littell *et al.* 1996, Stokes *et al.* 2000). A significant random effect was interpreted as a significant amount of variation among any given set of levels for such factor and not strictly as statistical differences between the levels chosen for that factor (the latter interpretation would be for a fixed factor effect; Herrera 2000).

## RESULTS

**SEED PREDATION ATTACK LEVELS.**—Overall, 65 percent of the plants sampled had at least one fruit attacked by the seed predator. On average, seed predators attacked  $28.9 \pm 2.3$  percent of fruits collected per plant and such value translated to roughly the same percent of seeds eaten, as each larva consumes all seeds within a fruit. Results from the regression models indicated lack of significance of the relationship between the log of population size and proportion of attacked fruits (linear model:  $R^2 = 0.04$ ,  $\beta = 0.2$ ,  $P = 0.4$ ; quadratic model:  $R^2 = 0.07$ ,  $\beta$  [linear] =  $-2.5$ ,  $\beta$  [quadratic] =  $0.81$ ,  $P = 0.5$ ).

Results from the mixed model analysis in GLIMMIX showed significant effects of region ( $F_{3,17} = 9.33$ ,  $P = 0.0007$ ) and population (estimate =  $0.2 \pm 0.1$ ,  $Z = 2.13$ ,  $P = 0.02$ ) on the proportion of attacked fruits per plant by seed predators. The effects of block and number of fruits per plant (covariate) were not significant (estimate =  $0.05 \pm 0.04$ ,  $Z = 1.36$ ,  $P = 0.09$  and  $F_{1,616} = 0.01$ ,  $P = 0.94$ , respectively). Differences between regions were as follows: the highest mean value was observed at the north region ( $45.1 \pm 2.5\%$  of fruits attacked; significantly different from all other regions:  $t \geq 2.2$ ,  $P \leq 0.05$  in all cases), followed by the center and south regions (which did not differ:  $29.1 \pm 2.4\%$  and  $26 \pm 2.4\%$ , respectively;  $t = 0.6$ ,  $P = 0.6$ ), and finally the east region, which

had a significantly lower mean value relative to all other regions ( $15 \pm 1.7\%$ ;  $t \geq 2.3$ ,  $P \leq 0.05$  in all cases; Fig. 1). The interpopulation pattern showed a great deal of variation in the mean value of proportion of attacked fruits, from  $60.1 \pm 5.5$  percent at northern population 1n to  $7.1 \pm 2.1$  percent at eastern population 25e. As indicated by the region effect results, the highest levels of damage were usually for northern populations, and although variable, damage levels were usually lowest for eastern populations (Fig. S2).

**PARASITOID ATTACK LEVELS.**—On average, parasitoids were found in  $24 \pm 1.8$  percent of fruits attacked by the seed predator. Results from the regression models indicated lack of significance for the relationship between the log of the number of seed predator-attacked plants and the proportion of fruits with parasitoids (linear model:  $R^2 = 0.03$ ,  $\beta = 0.16$ ,  $P = 0.4$ ; quadratic model:  $R^2 = 0.27$ ,  $\beta$  [linear] =  $5.27$ ,  $\beta$  [quadratic] =  $-2.13$ ,  $P = 0.06$ ).

Results from the mixed model analysis using GLIMMIX showed significant effects of region ( $F_{3,17} = 5.33$ ,  $P = 0.009$ ) and population (estimate =  $0.44 \pm 0.24$ ,  $Z = 1.81$ ,  $P = 0.03$ ) on the proportion of seed predator-attacked fruits with parasitoids. The effects of block (estimate =  $0.15 \pm 0.13$ ,  $Z = 1.14$ ,  $P = 0.12$ ) and proportion of fruits attacked by the seed predator ( $F_{1,399} = 0.14$ ,  $P = 0.7$ ) were not significant. Similar to seed predator attack levels, parasitoid attack also varied strongly across regions and populations. However, parasitoid attack intensity was consistently lower in the north (where seed predator attack levels were greatest), and in many cases greater in the center and especially the east (where seed predator attack was usually lowest; Fig. 1). Differences between regions were as follows: the largest mean value was observed at the eastern region ( $44 \pm 4.8\%$  of seed predator-attacked fruits with parasitoids), which, however, did not differ significantly from the center ( $29.7 \pm 4\%$ ) and south regions ( $24 \pm 4.1\%$ ;  $t \leq 1.6$ ,  $P \geq 0.08$  in both cases); the lowest mean value was observed at the north region ( $9 \pm 1.8\%$ ), which did not differ significantly from the

south region ( $t = -1.58$ ,  $P = 0.13$ ; Fig. 1). Interpopulation values ranged from  $65.1 \pm 10.5$  percent of fruits with parasitoids at eastern population 22e to 0 percent at southern population 19s. Overall, most eastern and central populations had the highest parasitoid attack levels, while northern populations had the lowest values (Fig. S2).

**PROPORTION OF SAVED SEEDS.**—Parasitoids saved on average  $4.0 \pm 0.4$  percent of all seeds produced by seed predator-attacked plants. Significant effects of region ( $F_{3,17} = 3.66$ ,  $P = 0.03$ ) and population (estimate =  $0.3 \pm 0.18$ ,  $Z = 1.66$ ,  $P = 0.04$ ) were observed on this response variable. The effect of block was not significant, although this result was marginal (estimate =  $0.16 \pm 0.1$ ,  $Z = 1.64$ ,  $P = 0.05$ ). Differences between regions were as follows: the largest mean values were observed at the eastern and central regions ( $6.8 \pm 1.1\%$  and  $5.2 \pm 0.8\%$ , respectively), which did not differ from each other or from the south region ( $4.3 \pm 0.9\%$ ;  $t \leq 1.91$ ,  $P \geq 0.07$  in all cases), while the lowest mean value was observed at the north region ( $1.4 \pm 0.3\%$ ), which did not differ from that of the south region ( $t = 1.62$ ,  $P = 0.12$ ; Fig. 2). As would be expected, the interpopulation pattern for proportion of saved seeds mimicked results for parasitoid attack levels: lowest for northern populations, consistently intermediate for central populations and, although variable, the highest values were observed for the eastern populations. Values ranged from as much as  $13.6 \pm 3$  percent of saved seeds at population 22e (eastern region) to 0 percent at population 19s (south).

## DISCUSSION

Overall, results from this study indicated spatial variation in the intensity of seed predator and parasitoid effects on the perennial herb *R. nudiflora*. Both seed predator and parasitoid attack intensity varied between regions and populations, and parasitoids had an

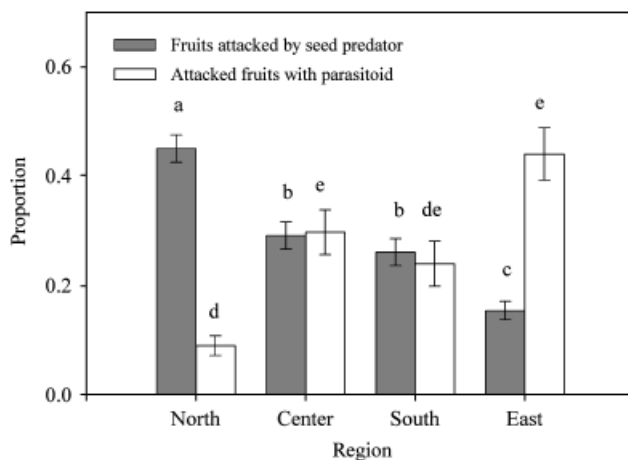


FIGURE 1. Proportion of fruits attacked by the seed predator and proportion of seed predator-attacked fruits with parasitoids for *Ruellia nudiflora* plants from each study region. Bars are means  $\pm$  SE. Different letters indicate significant differences between regions for each response variable separately ( $P < 0.05$ ).

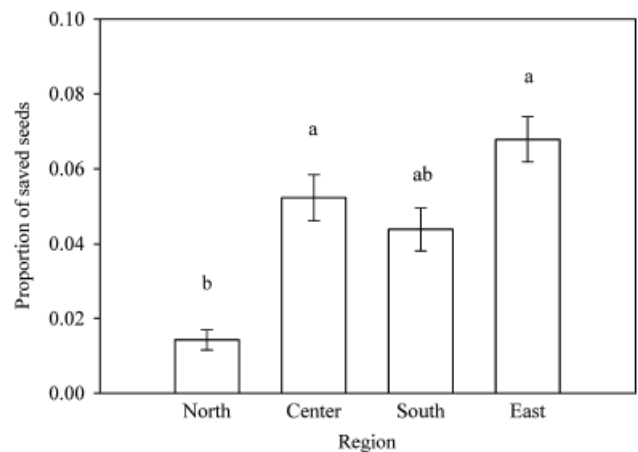


FIGURE 2. Proportion of *Ruellia nudiflora* seeds saved by parasitoids at each study region. Bars are means  $\pm$  SE. Different letters indicate significant differences between regions ( $P < 0.05$ ).

indirect positive impact on *R. nudiflora* (measured as the proportion of saved seeds), which also varied significantly across regions and populations; such finding represents evidence of spatial variation in the intensity of the trophic cascade under study. We consider this study is one of the few attempts to describe spatial variation in the intensity of direct and indirect effects for a tritrophic system.

**SPATIAL VARIATION IN SEED PREDATION INTENSITY.**—Seed predation on *R. nudiflora* averaged across regions and populations was of *ca* 30 percent of fruits attacked per plant (and *ca* 65% of all sampled plants had at least one fruit attacked). A recent review by Kolb *et al.* (2007) reported that predispersal seed predation levels from previous studies range from 0.2 to 100 percent, although most species show values  $\leq$  20 percent. This indicates that the average level of seed predation found for *R. nudiflora* was intermediate to high and may impact recruitment levels given that larvae eat all seeds within each fruit and that this plant most likely does not have a seed bank (Silvertown *et al.* 1993, Maron & Crone 2006, Kolb *et al.* 2007). In terms of spatial variation of seed predator impacts, both a region and a population effect were observed on the proportion of *R. nudiflora* fruits attacked by the seed predator (greatest attack levels in the north, and lowest in the east), indicating spatial variation in seed predator impacts on the plant at two spatial scales. Such spatially heterogeneous distribution of herbivore impacts appears to be a relatively common condition for predispersal seed predators (Kolb *et al.* 2007), as well as insect herbivores in general (Marquis 1992, Maron & Crone 2006).

The observed variation in seed predator attack levels across regions for *R. nudiflora* apparently was not related to plant population size, contrary to expectations based on findings from previous studies (*e.g.*, von Zeipel *et al.* 2006). Plant density, on the other hand, was similar between populations because it was one of the variables we tried to control during site selection, and is not expected to have influenced the results. However, additional factors such as abiotic conditions (climate; *e.g.*, Solbreck 1991), plant species composition (*i.e.*, availability of other host plants) and/or attack rates by the third trophic level (Gómez & Zamora 1994, van Loon *et al.* 2000; see discussion in next section) may have contributed to the observed pattern of seed predator attack intensity.

Regardless of the dominant factor(s) driving the observed spatial variation in seed predator attack intensity, these findings indicate very different scenarios of seed predator selection on *R. nudiflora* across populations, especially between eastern and northern populations, which may lead to divergent outcomes from this interaction between sites, and thus varying consequences on plant traits and/or performance (*e.g.*, flowering phenology, inflorescence height). Nonetheless, results presented here warrant further investigations that consider temporal variation in seed predation levels (*i.e.*, multiyear measurements; Sperens 1997, Ollerton & Lack 1998) to achieve a better understanding on the importance of seed predation on *R. nudiflora* reproduction and demography.

**EFFECT OF PARASITOIDS ON *R. NUDIFLORA* AND SPATIAL VARIATION IN TROPHIC CASCADE STRENGTH.**—Although we did not find a signifi-

cant relationship between proportion of fruits with parasitoids and number of herbivore-attacked plants at the population level, the quadratic regression model showed a negative value for the quadratic slope estimator and a marginal *P*-value, suggesting a tendency toward inverse density dependence of parasitoid attack rates. Factors such as constraints of females to detect sites with greater host number and/or quality, parasitoid aggregation and dispersal mechanisms, host handling time and constraints in parasitoid maximum attack rates may have been responsible for the apparent inverse density dependence pattern observed for parasitoid attack rates (Hassell *et al.* 1985, Stiling 1987, Walde & Murdoch 1988, Cronin 2003). However, regardless of which factors are involved in the observed pattern, this result should be taken with caution as the data used to calculate the regression included several parasitoid species and parasitoid community structure may vary across sites. These issues require more detailed studies that look at individual parasitoid species responses across *R. nudiflora* populations.

Previous studies have generally found weak indirect effects of carnivores on plant biomass or reproduction in terrestrial communities (Strong 1992, Polis & Strong 1996, Polis *et al.* 2000, Schmitz *et al.* 2000, Halaj & Wise 2001, Shurin *et al.* 2002). Accordingly, this study showed parasitoids had a mild positive impact on *R. nudiflora* seed production: 4 percent of seeds saved per (herbivore-attacked) plant on average. However, we also found a large amount of interpopulation variation in parasitoid effects on seed production, and in some cases the proportion of saved seeds was relatively high (*e.g.*, close to 14% for population 22e). Furthermore, parasitoid top-down control on seed predators may be particularly important for plants relative to top-down control on folivores, for example, because seed predators have a strong direct impact on plant fitness by killing new recruits, and thus may also influence plant population dynamics in a more straightforward manner (see von Zeipel *et al.* 2006).

Few studies have looked at spatial variation in carnivore indirect effects on producers, and most research on trophic cascades has been conducted at reduced spatial and temporal scales (but see Sinclair *et al.* 2000). Although recent meta-analyses such as that by Schmitz *et al.* (2000) have contributed to the understanding of variation in carnivore effects on plants (see also Halaj & Wise 2001, Shurin *et al.* 2002), studies at larger spatiotemporal scales are still lacking (Dyer 2007, Gripengberg & Roslin 2007). Interestingly, we found that seed predator and parasitoid attack levels associated with *R. nudiflora* exhibited a contrasting gradient of intensity across plant populations, a pattern that supports the inverse (but marginal) relationship suggested between parasitoid and seed predator attack levels. Specifically, northern populations were the most attacked by the seed predator but exhibited the lowest parasitoid attack levels, while eastern populations exhibited the lowest seed predator attack levels and the greatest parasitoid attack levels. This suggests that parasitoid top-down control on herbivores will be stronger for eastern populations. The observed scenario may be explained based on a large-scale parasitoid community-level perspective: both Ichneumonid and Braconid parasitoid species richness increases from north to east (and south) of Yucatan (A. González-Moreno, unpubl. data), with increasing forest diversity and

productivity, and previous studies have shown that top-down control on herbivores may be stronger at parasitoid species-rich sites due to more efficient host use by several parasitoid species (Hochberg 1996, Straub & Snyder 2008; but see Godfray & Waage 1991, Briggs 1993). This argument assumes that herbaceous vegetation at open sites where *R. nudiflora* occurs is influenced by parasitoid richness of adjacent forest patches (*i.e.*, ‘spill over effect’; *e.g.*, Thies *et al.* 2003); however, it fails to explain why parasitoid top-down control on herbivores is not equally strong in the south relative to the east, given that they share the same forest type (medium-height subdeciduous forest). Such inconsistency suggests that other biotic and abiotic factors such as climate and plant species composition at each site could also be contributing to the observed pattern.

Differences in parasitoid species composition across regions may have also been responsible for the observed differences between populations in seed predator and parasitoid attack levels, as well as in the proportion of saved seeds. Differences between parasitoid species in life history traits may result in them varying in their efficiency to mitigate herbivore impacts on plants (*e.g.*, Takagi & Hirose 1994, Wang & Keller 2002). For example, current taxonomic work has resulted in the identification of parasitoid specimens belonging to *Bracon* sp. and *Chelonus* sp. in *R. nudiflora* fruits, and species from these genera have contrasting life history strategies as the former includes species that are idiobiont and larval ectoparasitoids, which prevent any further development of the host after parasitization, while the latter includes koinobiont and egg-larval endoparasitoids, which allow the larvae to continue its development (Shaw & Huddleston 1991). Such differences in life history strategies may potentially determine how many seeds are eaten by herbivore larvae, as well as how many larvae are attacked per unit of time. Our current research is focused on identifying the parasitoid species found in *R. nudiflora* fruits, as well as addressing spatial differences in the structure of the parasitoid community. Both aspects will greatly contribute to understand the observed patterns in this study.

EVOLUTIONARY IMPLICATIONS OF SPATIAL VARIATION IN PARASITISM INTENSITY.—Finally, it is important to mention some of the potential evolutionary consequences of spatial variation in parasitoid attack rates. Assuming that the observed pattern remains relatively consistent in time (as supported by preliminary data; V. Parra-Tabla, unpubl. data), then parasitoid top-down control on herbivores may act as a selective force on timing of seed predator attack, as well as weaken seed predator selection on plant traits. For example, seed predator selection on flowering time has been shown for many plant species (see review by Elzinga *et al.* 2007), including *R. nudiflora* (P. Carbajal-Borges, unpubl. data). However, strong top-down control of parasitoids on herbivores may dampen seed predator selection on plant reproductive phenology as would be expected for some eastern populations in this study. In contrast, northern populations would be expected to be more strongly selected for flowering time by seed predators given a weaker effect of parasitoids.

CONCLUSION.—We found spatial variation in the intensity of interactions involving *R. nudiflora*, a Noctuid seed predator and its

associated parasitoid fauna both within and between regions throughout the studied landscape. The strength of the trophic cascade varied strongly across sites: it was greatest for eastern populations and negligible for most northern populations. The overall pattern suggests that some locations may represent ecological and (potentially) evolutionary hotspots for which the strength of second or third trophic level effects is greatest (*sensu* Thompson 2005), resulting in divergent net effects on *R. nudiflora* fitness measures and population dynamics. Nonetheless, a long-term temporal characterization of second and third trophic level effects on *R. nudiflora* is needed. Such information is necessary to determine the ecological and evolutionary implications of the observed effects for this tritrophic system. We consider that future studies using this system should include: (1) manipulations of second and third trophic level abundances; and (2) evaluate the effect of other biotic (plant species composition and richness) and abiotic (climate) factors on insect abundance and attack rates on *R. nudiflora*. Finally, further studies that look into detail at the characteristics of the parasitoid community (*i.e.*, guilds, richness) associated with *R. nudiflora* and how they change across space will most likely help explain the observed patterns.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

FIGURE S1. Location of *R. nudiflora* populations sampled across the state of Yucatan.

FIGURE S2. Proportion of fruits attacked by the seed predator and proportion of seed predator attacked-fruits with a parasitoid for *R. nudiflora* plants at each study population.

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