Distance-dependence in vertebrate and invertebrate seed predators: A case study on Aleppo pine (*Pinus halepensis*)

by Omri FINKEL

Seed predation is an important factor affecting the recruitment of plants. Several hypotheses have been suggested as to how and if does seed density affect seed predation. This study, using the placing of experimental seed plots, with and without vertebrate and and invertebrate predators, in and around an Aleppo pine stand, at different tree densities, analyses the relation between predation rates and distance from plants.

Introduction

In an attempt to explain the high biodiversity in areas such as the tropics, a model was proposed independently by D.H. Janzen and J.C. Connell (JANZEN, 1970; CONNELL, 1971). This model explained the observed spatial distribution of conspecific plants by density-dependent seed predation. It proposes that whereas the density of dispersed seeds declines with distance from the parent tree, the probability of predation declines with distance as well, because specialist seed predators tend to concentrate their efforts near the parent tree where the seed flux is greater and therefore prey is denser. These two factors create a population recruitment curve that reaches its peak at a distance from the tree where the probability of survivorship, multiplied by the seed-density, is highest. This acts to prevent clumped distribution of single species, leaving room for other species to establish amongst it.

Although the declining with distance seed-shadow component of this model is widely accepted (HAMMOND & BROWN, 1998), the predation and survival components are not. A number of models alternative to the J-C (Janzen-Connell) model have been proposed. It has been suggested that while predation rate does decline with distance, this change does not suffice to compensate for the decrease in seed density with distance, thus producing a recruitment curve that continuously

declines with distance (HUBBELL, 1980). In fact, seed predation acting in concert with seed flux can create a wide range of establishment patterns (MCCANNY, 1985, NATHAN & CASAGRANDI, 2004). Where survivorship rises exponentially in the exact rate as seed flux declines, recruitment level will remain equal with distance. Survivorship may also actually decline with distance, due to factors such as predator satiation near the parent plant.

Many attempts have been made to test the predictions of the J-C hypothesis in a wide variety of habitats and conditions, usually testing whether the predation rate declines with density/distance. Standing alone, this component is referred to as the "escape hypothesis" (HOWE & SMALLWOOD, 1982). It should be noted that a test of this hypothesis can offer support to the J-C model but cannot differentiate it from alternatives such as the Hubble model (HUBBELL, 1980), as both models assume increasing survival with distance. Many of these results were summarized in reviews (CLARK & CLARK, 1984; HAMMOND & BROWN, 1998) which yielded mixed conclusions. In a recent meta-analysis, HYATT *et al.* (2003) collected data from 40 studies to compare predation of seeds and seedling survival at different distances from trees. They found no correlation between the distances from the parent and the probability of predation.

Notwithstanding, density-dependent seed predation may be found under certain environmental conditions rather than others or with certain types of predators. Exclusion

Picture 1:
The study site

experiments are an important tool in understanding how different types of predators effect plant recruitment as well as for learning of the role different predators play in shaping recruitment patterns in the same habitat. Experiments such as these were excluded from the meta-analysis (HYATT *et al.* 2003) on the grounds of not describing the overall predation rate. Characteristics of the predator have been shown to determine density-dependence (HAMMOND & BROWN, 1998). Hammond & Brown grouped studies according to predator types, showing that non-vertebrates seem to be more density-dependent than vertebrates. This difference was explained by the larger home range of vertebrates relative to the distance of prey dispersal, and by the tendency of vertebrates to be more generalists.

Another question that should be dealt with is the question of scale. If seed density at different spatial, as well as temporal scales, affects the seed predation rate, it is important also to assess at what scale does this effect take place. This issue holds great importance for the spatial design of an experiment testing predation rates.

I addressed these questions by conducting an experimental predation field study as a part of a larger population dynamics study currently underway in at Mt. Pithulim in the Judean Hills, Israel (NATHAN, 2004). The predation rate of Aleppo pine (*Pinus halepensis*) seeds was measured at different pine densities using experimental methods.

The aim of this study is to determine the rate of predation on pine seeds of different predators on Mt. Pithulim, and to find whether correlation exists between pine density and the rate of seed predation. The main non-vertebrate predators in the current study are ants, present in different nests in and out of the main stand. While each nest may have a smaller home range than the distance of dispersal, the general distribution of ants around the study site is greater than the pine distribution — ants can be found where there are no pines. This suggests that ants should not be expected to act as other invertebrates do (HAMMOND & BROWN, 1998). Contrary to the findings of HAMMOND and BROWN (1998), density-dependence should be expected of the vertebrate predators in this case, as they are likely to concentrate their efforts on high quality prey such as pine seeds.

Another goal of the study was to test the effect of the number of seeds in a patch; the fate of a single seed is compared with the fate of a patch of several seeds. The question asked is whether patches with a large number of seeds are more easily detected. It was expected that plots with a larger number of seeds will be detected earlier. Another question that is addressed is the question of scale, temporal as well as spatial: on what spatial scale do different predators sense a difference in the density of their prey, and on what time scale is their effect more apparent. It was expected that the response of predators to tree density will be affected by the scale of density and that the response would be time dependent.

This is achieved by placing seed plots at a large number of locations complying with different categories of tree density, measuring the rate of seed disappearance and statistically analyzing the gathered data.

Methods

The study site

The site is a 60 ha plot including an Aleppo pine stand, embedded within a *Quercus-Arbutus* maquis and *Sarcopoterium Cistus* batha. All pine trees in the site have been previously mapped and detailed orthophotos are available. Data regarding seed flux is regularly collected using seed traps placed at 54 sites in the study area. The two main seed predators that have been observed on site are ants and broad toothed mice (*Apodermus mystacinus*). It is also reasonable to assume that birds are responsible for at least a small portion of the seed predation.

Seed predation experiment

Experimental plots were placed in 89 locations in the area, stratified over different tree densities at different scales, selected by measuring the tree density (the number of trees at three concentric radii from each 1x1 meter grid cell in the study area: 10, 30 and 50 meters. Then, grid cells were classified according to tree density on these three different scales. For example, if a grid cell was in a small, dense, isolated pine patch, it would be classified as "dense-sparse-sparse"

Category	Description	Total number of plots	Single seed plots	Multiple seed plots
s-s-s	sparse at all ranges	21	17	4
d-s-s	dense at close range only	16	13	3
s-s-d	dense at long range only	10	8	2
s-d-d	sparse at close range only	17	13	4
d-d-d	dense at all ranges	25	20	5
Total		89	71	18

Table 1:
The number of plots in each category

or "d-s-s". "Dense" was determined as 6-25 trees for the 10 m radius (0.019-0.08 trees/m2) 30-150 trees for the 10-30 m ring (0.012-0.06 trees/m²) and 60-250 trees for the 30-50 m ring (0.012-0.05 trees/m²). "Sparse" was: 0-3 trees for 10 m (0.0-0.01 trees/m²), 0-15 trees for 10-30 m (0.0-0.006 trees/m²) and 0-30 trees for 30-50 m (0.0-0.006 trees/m²). The densities were calculated using Arcmap GIS software (ArcMap 9.2, ESRI, Redlands, CA) and care was taken to make sure that each category includes at least 5 plots. Out of the eight possible categories, the chosen sites included the following five: "d-d-d", "d-s-s", "s-d-d", "s-s-d" and "s-s-s". The three remaining possibilities "d-d-s", "s-d-s" and "d-s-d" did not appear in enough grid cells to be included. This does not impair the study as the five included categories can be scaled from sparse to dense in all three radii.

The sites were chosen in a way that each one of the 54 seed traps on site had an experimental plot near it. This will enable the integration of seed flux data with the data gathered in this study. The total number of categorized plots was 89 (See table 1).

Every plot had 4 seeds placed in it, one only for each treatment, so as not to artificially increase natural seed density (BLATE *et al*. 1998). The predation rate was calculated from the time each seed survived. The treatments were designed to exclude, and therefore separate, different potential predators.

The seeds that were used in the experiment were extracted from the cones by heating for five minutes at 100°C, which causes the cones to open and inhibits germination. They were separated from their wing and red spray paint was applied to them to facilitate their detection.

In order to exclude the rodents, a seed was placed in a 20X20 cm plastic cage. This treatment will be designated "ant predation". In order to exclude the ants, a seed was glued to a 15 cm string that was tied to

the cage and placed outside of it. This treatment will be designated "rodent predation". Another seed was simply placed outside the cage to measure total seed loss ("untreated") and a fourth, "control" seed, was placed inside the cage and glued to a string.

Preliminary experiments were conducted with ants, caged mice and broad-toothed mice in the field and confirmed that each exclosure worked for one type of predator and not the other and that the glue, spray paint and heating did not affect predator preference.

In 18 out of the 89 plots, five seeds were placed for each treatment, reaching a total of 20 seeds per plot, rather than four in the regular plots. These will be called "quintet" plots.

The plots were inspected throughout the first two weeks of the month of August 2006, daily at first and later every few days (points on fig. 3 represent inspections) until the predation curve reached a plateau. In total, the plots were examined 11 times during the study period. 10 examinations were done during the first 15 days, and the last one 60 days after the initiation. The plots were then inspected once more in October when they were collected. A seed that had disappeared was not replaced. This, coupled with the use of only one seed per treatment and measuring its survival time, allows us to avoid creating a large seed reservoir, thus altering the natural seed shadow (Blate, 1998).

The raw data gathered in this experiment was treated in different ways. Two approaches were applied in order to compare predation rates in different categories. One is the comparison of the total percentage of surviving seeds at different points in time with special attention given to day 15 and day 60. This was done simply by counting how many seeds have survived for each category and every treatment and dividing this by the initial number for every category and treatment. To find whether the difference between categories was significant, a χ^2 test was preformed, the null model of which being the number of survivors that would have been in each category had the predation rates been uniform. The second approach was to compare the mean survival time (the time elapsed from the study initiation till the seed disappeared) for each category. These averages were then compared to find a significant difference using ANOVA.

These approaches are both based on the assumption that the earlier the seed disappears, the higher the predation rate. The above analyses were preformed separately for single-seed and quintet plots. These two types of plots were then compared using a paired 1-tailed t-test for the elapsed time till a seed was predated where every quintet plot was paired with its closest neighbor of the same category, as well as by calculating the percent of predated seeds for each plot type.

Confounding effects

In order to single out any additional effect on predation rate, several more plots were placed in identical densities. Ten were placed under tree canopies, 10 on bare ground or rock and 10 under a bush. This enabled the comparison of factors other than density that might have affected the predation rate.

In addition, all 89 experimental plots were characterized by percent vegetation cover and the different plant species in a 2X2 m square around the plot.

Results

Comparing the survival rates in single seed plots at day 15, the control seed (string + cage) has somehow disappeared in a few cases (See fig. 1a). Predation of seeds available only to ants (See fig. 1d) was not significantly different between categories (χ^2=4.69, df=4, P=0.32). Predation of seeds available only to rodents (See fig. 1c), however, exhibited density dependence, mainly at the long-range radial ring of 30-50 meters (χ^2=14.06, df=4, P=0.007). The overall predation rate, demonstrated in fig. 1b, shows a similar trend (χ^2=12.13, df=4, P=0.016), due to the fact that predation by rodents is much more extensive than predation by ants.

Looking at the same setup after 60 days, two observations stand out. One is the fact that all but four of the untreated seeds have disappeared (See fig. 3b) and the other is the fact that the trend that was visible on day 15 does not exist any more and predation by neither ants nor rodents is density dependent (See fig. 3c,d). A χ^2 test yielded no significant density effect in any of the treatments.
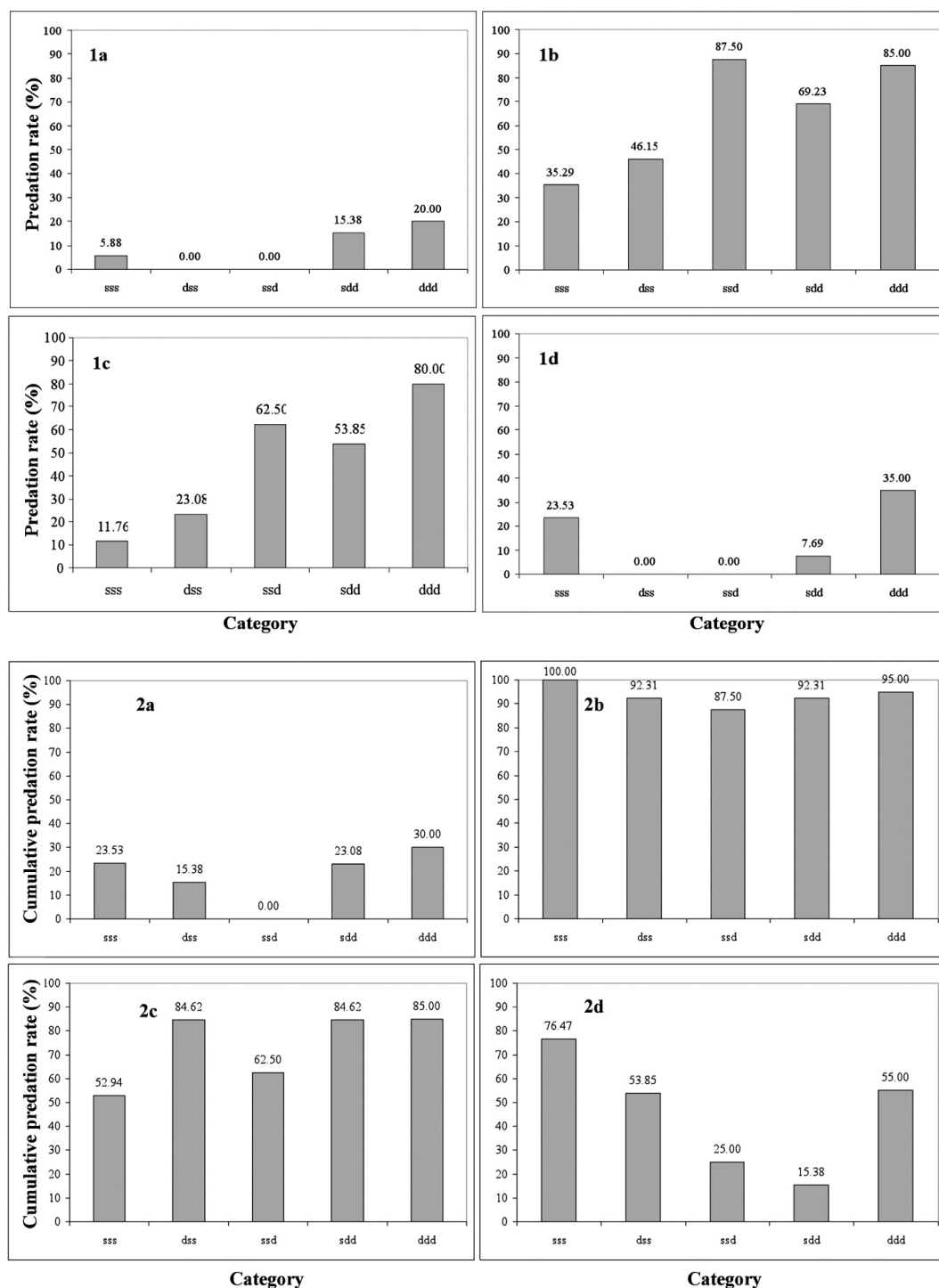
Figure 3 displays the change in predation with time. Between days 10 to 15 the distribution of seed predation seems to have reached a steady state in most cases. At day 60 it is apparent that a plateau was not reached after all and that the distribution of predation rates has also changed.

A second analysis displays the average survival time of seeds in each of the density categories. This analysis includes all time points. An obvious problem here is that for some seeds, the ones that had survived longer than 60 days, the actual survival time is not known. To solve this, two extremes were taken into account. One is that all of these seeds have disappeared on day 61, right after the last sampling, and the other is that all had survived throughout the season, for which case they were assigned an arbitrary survival time of 100 days.

The results of this analysis for single seed plots show a significant difference in preda-
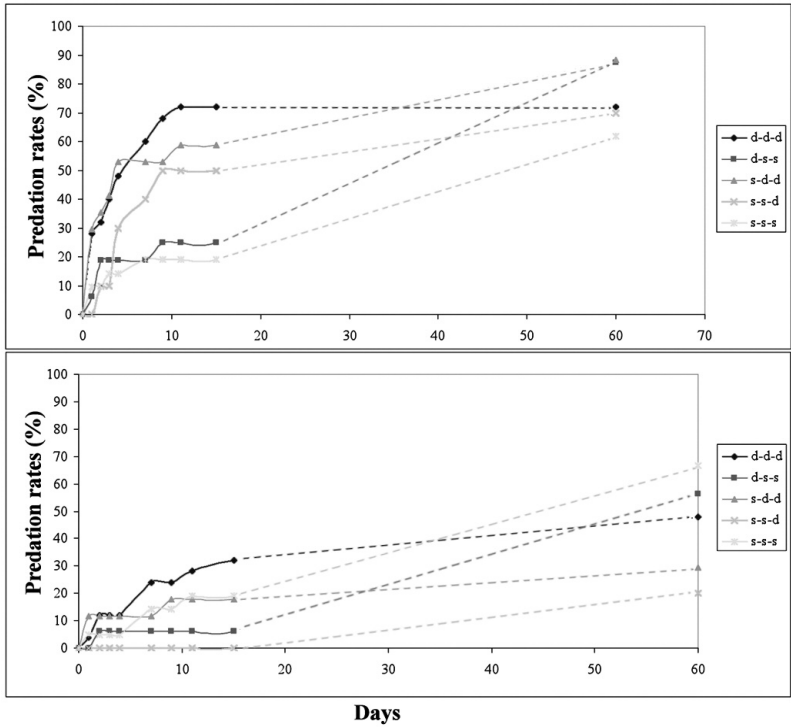
Fig. 3:
Predation rates with time for ant (top) and rodent (bottom) exclusions, in different categories. Note the change from day 15 to day 60, especially for d-s-s. The broken line represents the long period between samples. This plot includes both single seed as well as quintet plots.

Fig. 4:
Comparison of mean survival time in the different density categories both for ant (top) and rodent (bottom) predated seeds. These graphs display the dataset in which the maximal survival time was set to 100 days.

tion by rodents between density categories (See tables 1,2). A post-hoc analysis shows a significant difference between categories s-s-s and d-s-s- to d-d-d (Pddd-sss<0.001, Pddd-dss=0.006, see Appendix 1). The same analysis for ant predation shows a significant difference between groups (See fig. 4 top, table 2), but no clear relation with the density was detected. Using this method, the overall predation rate was significantly different between categories only with the maximum survival time set to 61 days (See table 2).

In the 18 plots in which five seeds of each treatment were placed, no density dependence emerges (See fig. 5). A paired t-test showed no significant difference between single seed and quintet plots (t=0.8, P=0.217).

Other factors that were measured, such as canopy cover, did not show a significant influence over predation rates.

The effect of factors other than seed density was analyzed by comparing predation rates at different environments at similar seed densities. A χ^2 test showed no significant difference between these environments (See table 3).

Discussion

The results show a significant correlation between pine density and predation of seeds by rodents (See fig 2d, 4). No other significant correlation was found. Other environmental factors did not show correlation with predation rates.

The correlation found does not imply causality. It is not clear whether these rodents are reacting to the density of the prey or to some other factor, such as better protection from predators or a slightly cooler climate under the canopy cover of the dense pine stand. Albeit, it should be noted that the sparse areas are not barren, but covered with batha plants at varying densities. The experiment entitled "confounding effects" described above is an attempt at isolating several environmental factors other than density that might affect predation. However, this examined only variance in a small spatial scale and only of three parameters. Other factors that were not examined may have an influence. For instance, it is
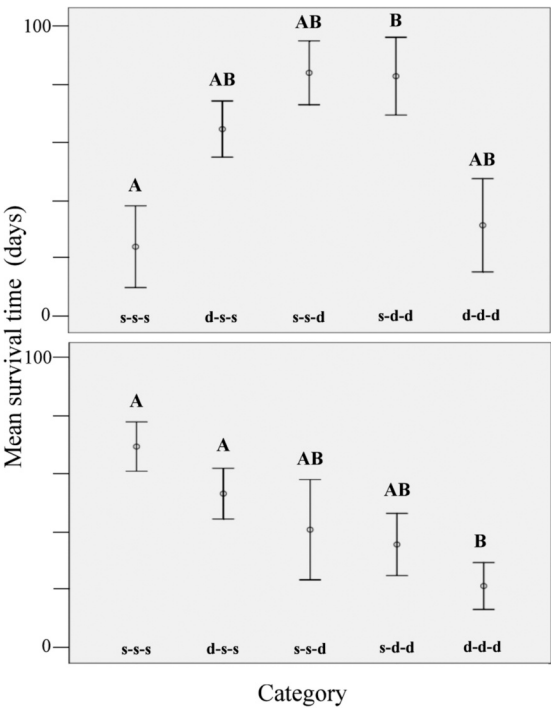
possible that rodents spend more time in the dense stand as protection from predation by birds of prey, or simply in order to avoid the sun heat, as this area is not affluent with water sources.

A survey of the demography of ants and rodents in the study area is a possible approach towards clarifying some of these questions.

The question why are rodents responsible.

Time scale

Interestingly, the distribution of predation rates between categories at day 60 differs greatly from that of day 15 (See fig.3). This observation emphasizes the importance of using the appropriate time scale when conducting such an experiment. While figures from day 60 provide valuable data as to the great extent of predation throughout the season (See fig. 2b), they do not provide sufficient resolution. When all plots were paid a visit by a predator, it is not possible to determine which plot was visited first. On day 15, on the other hand, predation was still partial, which enables differentiation of predation rate between categories. This rationalization does not explain why after supposedly reaching equilibrium between days 10 and 15, something has changed during the next 45 days and predation increases once more. This implies that the foraging effort is not uniform throughout the season. Perhaps the effort increases as the cold season approaches. These questions, left unanswered, are a possible topic for further study about how does a predators annual life cycle affect its foraging effort.

Spatial scale

The correlation between predation by rodents and pine density did not appear at all radii. In fact, as seen in figure 4, the category d-d-d is significantly different only from categories s-s-s and d-s-s. The latter are not significantly different from one another. This demonstrates a lesser effect of the close radius (10 m). The category "d-s-s" includes cases of isolated groups of pines away from the main stand. Such a patch is apparently not more attractive to a rodent than its surroundings. Therefore, conducting such an experiment at a single range might be misleading, as a result of using the wrong spatial resolution. One of the factors that could determine at what range is density depend-

Treatment	df	61		100	
		F	P	F	P
Predation by ants	70	3	0.024	3.6	0.01
Predation by rodents	70	5.95	<0.001	4.23	0.004
No treatment	70	3.3	0.016	1.99	0.106
Quintet	17	0.77	0.564	0.79	0.553

ence apparent is the predator's home range. This data is lacking in the present study.

Comparing mean survival time between categories, arbitrary values were assigned as 61 and 100 days (see methods). Results are significant for both. Surprisingly, using this analysis and contrary to the results of the isolated analysis preformed on days 15 and 60, survival for seeds taken by ants is significantly different between categories, although clearly not consistent with density levels.

This suggests that ant nests are not distributed randomly throughout the area and that another environmental factor is influencing this distribution.

Conducting a demographic survey in the study area of the home range of ants and rodents, and of the relation between home range and predation patterns is a possible course of action for further research, in attempt to answer these questions.

Table 2:
Values of ANOVA statistics for the difference between the five density groups (s-s-s, d-s-s, s-s-d, s-d-d, d-d-d) with different treatments and with the maximal survival time set to 61 or 100 days.

Table 3:
Results of χ^2 test of the difference between three different environments: open, under a bush and under a tree canopy.

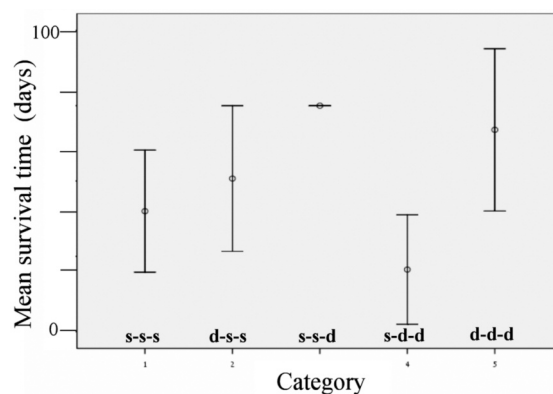|	no treatment		predation by rodents		predation by ants		cont.	
days	15	60	15	60	15	60	15	60
χ^2	4.04	4.80	1.38	0.93	0.58	0.30	2.99	5.50
df	2	2	2	2	2	2	2	2
P	0.13	0.09	0.50	0.63	0.75	0.86	0.22	0.06

Fig 5:
Comparison of mean survival time for quintet plots in different density categories.

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Number of seeds in a plot

A more intuitive way to conduct this study would have been by placing a several seeds at every site, measuring the rate of predation by counting the number of remaining seeds at each site at different points in time. This method was not used so as not to create an artificially dense spot. The behavior of rodents detected in the field was that if a group of seeds was found they were all taken immediately.

When analyzing quintet plots separately, no trend emerges. Also, it was observed that of the seeds exposed to rodents, all five disappeared at the same time. The fact that the paired t-test showed no significant difference between single and quintet plots, suggests that had the quintet plots been more numerous, they might have yielded similar results as did the single seed plots. An additional comparison, with a larger number of repeats is necessary in order to properly answer this question.

In conclusion, this study supports the hypothesis that the predation by certain predators (rodents) is correlated with seed density, what may lead to J-C dynamics. It also demonstrates the importance in the use of tools such as predator exclusion, as ants and rodents react differently to density; and the inspection of several spatial and temporal scales, as different patterns emerge in different scales.

O.F.

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Summary

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Seed predation is an important factor affecting the recruitment of plants. Several hypotheses have been suggested as to how and if does seed density affect seed predation. This study, on Aleppo pine, is a test for the prediction made by Janzen (1970) and Connell (1971), that predation rates decrease as distance from a conspecific plant increases. The method used is the placing of experimental seed plots, with and without rodent and ant exclosures, in and around an Aleppo pine stand, at different tree densities. Density/distance-dependence is supported for rodents, but not for ants. No significant difference was found between plots with 20 as opposed to 4 seeds.