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An Experimental Demonstration of Density-Dependent Reproduction in a Natural Population of *Diamorpha smallii*, a Rare Annual

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Summary. In a North Carolina population of *Diamorpha smallii*, densities were artificially manipulated by thinning stands to predetermined densities. Individuals in areas of high density produce fewer seeds than individuals at low density in the same habitat. Three components of fecundity, the number of flowers per plant, the number of fruits per plant, and the number of seeds per fruit, were negatively correlated with density. The relationship of components of fecundity to density were used to develop a model of population regulation for this species. These results and predictions from the model are discussed in relation to other density studies.

Introduction

Interest in the effects of density upon natural populations, particularly how density influences an individual's survival and fecundity, orginated with the controversy concerning the regulation of population numbers (Andrewartha and Birch 1933), but despite numerous studies of density in experimental and crop situations, we have relatively little direct evidence of density reponses in natural plant populations (Antonovics and Levin 1980). Results from experiments conducted in artificial environments have led to the general conclusions that high densities cause increased mortality rates and positively skewed size distributions in plant populations (Harper 1977). These observations have led to the law of constant final yield (Shinozaki and Kira 1956); that is, at initial densities higher than the minimum threshold (particular to a given species in given conditions), the final total biomass per unit area is independent of density. As a result of stunting and/or mortality, the limiting yield is not exceeded.

While the general form of density responses in artificial plant populations has proven remarkably consistent for a wide range of species and conditions, it is not valid to generalize results from such experiments to natural populations. The natural heterogeneity of physical factors and interactions with other organisms are likely to affect the responses to density of demographic characters that determine population growth. Furthermore, observational studies of populations in nature have the inherent problem that density and site effects are confounded. Antonovics and Levin (1980) discuss this and other problems inherent in the observational studies which preclude definitive determination of the effects of density. Experimental field studies are needed to further our knowledge of the role of density in the regulation and evolution of plant populations (Murdoch 1970). The few studies that have been done (Watkinson and Harper 1979; Inouye 1980) have confirmed the importance of density-dependence in natural plant populations using experimental approaches.

In this paper, we report the results of an experiment in which densities of *Diamorpha smallii* were altered early in the life cycle and the reproductive response quantified in a natural population. We designed our research to answer the following questions:

1) Is there a causal relationship between density and the amount of reproduction per plant in a natural population of *Diamorpha*?

2) Do plants in naturally varying densities show the same relationship between reproduction and density? If not how do they differ?

3) Do three components of fecundity (number of flowers per plant, fruits per plant, and seeds per fruit) all have the same relationship to density in unmanipulated plots?

4) At what distances do individuals affect one another's fecundity?

Materials and Methods

Diamorpha smallii, a succulent winter annual in the Crassulaceae, is known only from a series of granite outcrops that occur sporadically in the southeastern United States (Wilbur 1964). This species has been considered a member of the genus *Sedum* (Sharitz and McCormick 1973; Radford et al. 1968). However, we follow Wilbur (1964), who reviewed the taxonomic evidence and restored *Diamorpha* to the status of a distinct monospecific genus.

Depressions in the rock surface accumulate soil that supports a variety of plant species, many of which are endemic to granite outcrops (McVaugh 1943; Wyatt and Fowler 1978). Species typically occur in concentric rings corresponding to a soil depth gradient within a single depression (McVaugh 1943). Pure stands of *Diamorpha smallii* occupy the outermost ring where the soil is the shallowest, although occasionally it occurs in deeper soils with a few other species (Sharitz and McCormick 1973). In *D. smallii*, seeds germinate in late fall or early winter; seedlings overwinter as small rosettes and then resume growth early the following spring. The plants flower in April and May (Radford et al. 1968), and seeds are mature by the end of June. Wyatt (1981) reported that individuals are self-incompatible and are pollinated primarily by ants. The seeds possess no special dispersal mechanisms, rather they are dispersed over the outcrop by flowing water (Chapman 1977; Sharitz and McCormick 1973).

In order to study the effect of density on reproduction in *D. smallii*, a natural population was followed over the course of a growing season at the Overton outcrop located in Franklin Co., North Carolina (see Palmer 1970, for a description of the site). In March, 1980, while the plants were in the rosette stage, densities of *D. smallii* were artificially manipulated by thinning extant stands to five different densities, in which plants were evenly spaced in a square grid pattern (20 cm × 20 cm). The five spacings were 8, 4, 2, 1, and 0.5 cm between plants,

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which correspond to densities of 1.56, 6.25, 25, 100, and 400 plants per dm^2 respectively. Included also was a control whose numbers were unaltered but counted. The treatments were randomly assigned to contiguous plots within a single site (each site was located in a distinct depression in the rock surface). Treatments were set up in six different sites. In this manner, a two-way factorial design of site crossed with density was established. There was no replication within sites. Plants were thinned carefully with tweezers in order to minimize disturbance. At the peak of the flowering season in May, 1980, all living plants were removed from the 1.56, 6.25, and 25 density plots, while from the 100, 400 and controls, a subsample of 1 dm^2 was collected. The number of surviving plants was determined for each plot, and the difference between initial and final numbers was considered mortality. The number of flowers produced on every plant was counted and recorded. The depth of the soil was also determined for each plot in five locations by pushing a knife into the soil until it hit the underlying rock and measuring the depth. The average of the 5 measures thus obtained for each plot was used in further analyses.

In addition to the experiment just described, we made collections of 41 undisturbed 1 dm^2 plots at two dates: in May, while the plants were flowering, and in July, when the fruits were mature. All plants within each plot were removed and the number of flowers, or fruits, on each individual was determined. In addition, from the second collection, 10 fruits from each plot were sampled and the number of seeds in each determined. Soil depths were determined for each plot in the same manner. Values for density, number of flowers per plant, fruits per plant, and seeds per fruit were log transformed (base 10) in order to meet the assumptions of normality for the statistical analyses. All analyses were carried out on mean response per plot, since it is to be expected that the responses of individuals within a plot are not independent.

Table 1. ANOVA of log mean flowers per plant in experimental plots

Source	DF	SS	MS	F	pF
Site	5	0.212	0.0424	8.25	0.0001
Treatment	5	0.330	0.0660	12.86	0.0001
Error	24	0.123	0.0050		
Total	34	0.665			

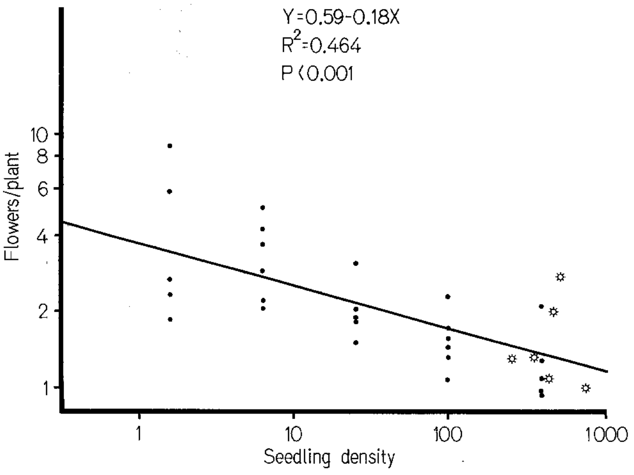

Fig. 1. Regression of log flowers per plant versus log seedling density (plants per dm^2) in the experimental plots. Stars represent controls

Results

In the plots where density was experimentally manipulated, both site and density were highly significant ($p<0.0001$) determinants of flower production (Table 1). The absolute flower production varied from site to site, but the direction of the density response was the same for each treatment within a single depression (Table 2). The regression of log flower number on log density was negative and highly significant both for initial (rosette) density and final (flowering plant) density (Fig. 1 and Fig. 2). The frequency distributions of number of flowers per plant (Fig. 3) show that at high densities the majority of plants produced only a single flower, and at lower densities most plants produced more than one flower, with many plants producing over 10 flowers. These frequency distributions differ significantly from a normal distribution ($P<0.01$) in all experimental treatments. Each of the 6 distributions is positively skewed, with skewness increasing with increased density.

While there appeared to be a strong trend of increased mortality with increased density (Fig. 4) these data were not statistically analyzed, because we had little confidence in the accuracy of our mortality estimates. In some plots final density exceeded initial density. This could have come about by regeneration of incompletely removed plants or by inaccuracy of our counts.

Samples from the 41 undisturbed plots displayed a similar response to density (Fig. 5). The regression of log flower number on log density was again negative and highly significant. The depth of the soil was also negatively correlated with the log flower number ($r=0.23$, ns). Soil depth and density were not significantly correlated ($r=-0.0052$, ns).

Table 2. Mean flower number per plant ± standard error for the experimental plots. Sample sizes in parentheses

Site	Treatment					
	C	0.5	1	2	4	8
1	1.31 ± 0.038 (515)	1.29 ± 0.029 (428)	1.58 ± 0.054 (249)	2.04 ± 0.100 (148)	3.72 ± 0.306 (46)	2.71 ± 0.491 (17)
2	1.10 ± 0.024 (474)	0.96 ± 0.033 (81)	1.47 ± 0.059 (104)	1.87 ± 0.142 (56)	2.20 ± 0.182 (49)	1.87 ± 0.295 (8)
3	1.35 ± 0.044 (350)	0.97 ± 0.030 (239)	1.09 ± 0.051 (202)	1.89 ± 0.121 (80)	2.92 ± 0.240 (36)	2.68 ± 0.265 (19)
4	0.99 ± 0.007 (582)	1.10 ± 0.043 (50)	1.33 ± 0.047 (243)	1.52 ± 0.070 (121)	2.09 ± 0.182 (32)	2.33 ± 0.333 (3)
5	2.01 ± 0.116 (112)	1.29 ± 0.037 (353)	1.72 ± 0.072 (276)	–	4.29 ± 0.394 (51)	5.94 ± 0.957 (17)
6	2.73 ± 0.129 (154)	2.11 ± 0.152 (84)	2.31 ± 0.192 (49)	3.13 ± 0.201 (105)	5.16 ± 0.536 (45)	9.00 ± 1.55 (9)

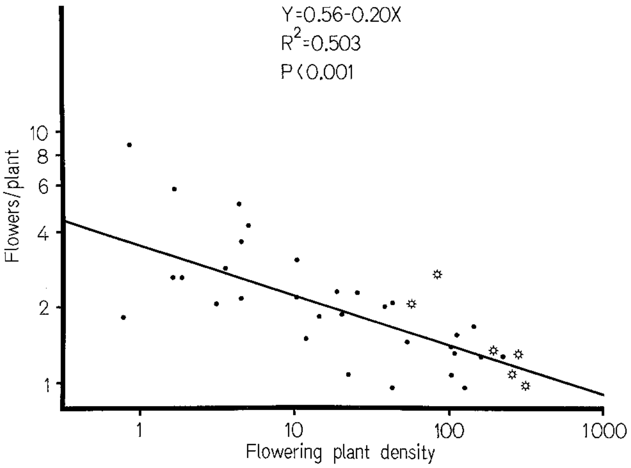

Fig. 2. Regression of log flowers per plant versus log flowering plant density (plants per dm^2) in the experimental plots. Stars represent controls

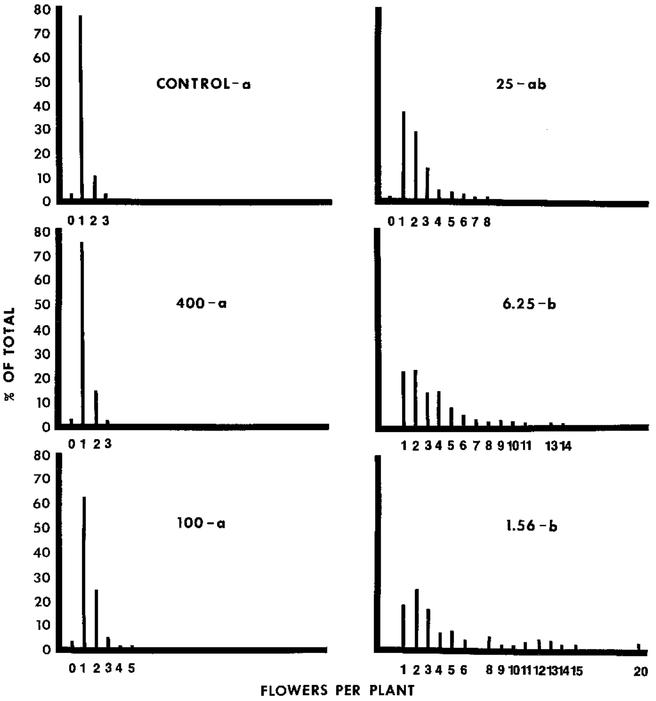

Fig. 3. Frequency distributions of flowers per plant in each density treatment. Treatments are lumped over all sites. Different letters signify significant differences between treatments

The regression of log fruits per plant on log density for the 41 plots sampled in July was also significantly negative (Fig. 6), but the relationship was not as strong ($R^2=0.490$) as that between log flower number and log density ($R^2=0.659$) earlier in the season. The slopes for flower and fruit production were not significantly different from each other, but both were significantly different ($p<0.05$) from the slope of the regression from the experimental plots. Soil depth was not correlated with fruit production or density.

Fruits contained an average of 7.35 seeds (Table 3). The regression of the log number of seeds per fruit and log density showed a weak negative relationship ($Y=1.027-0.08X$; $R^2=0.065$; $p=0.10$). The number of seeds per fruit was not correlated with soil depth.

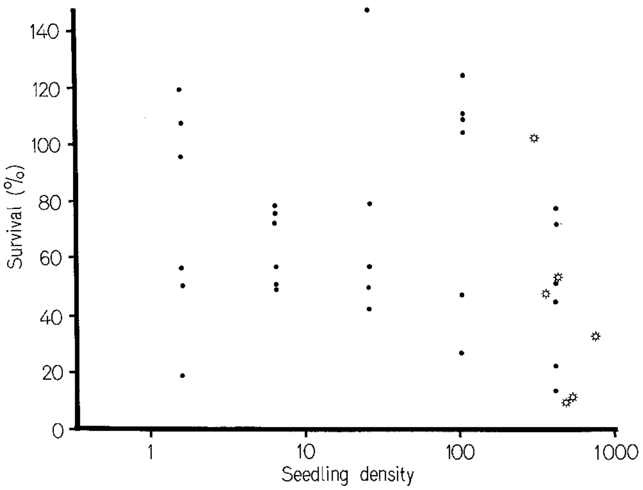

Fig. 4. Scatter plot of mortality in experimental plots versus plant density (plants per dm^2). Stars represent controls

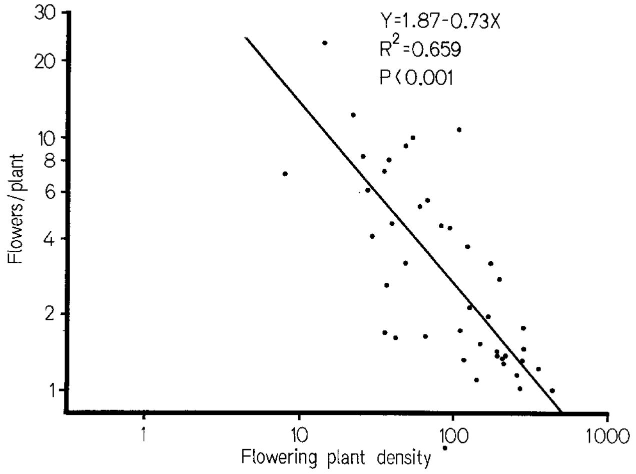

Fig. 5. Regression of log flowers per plant versus log flowering plant density (plants per dm^2) in 41 undisturbed plots

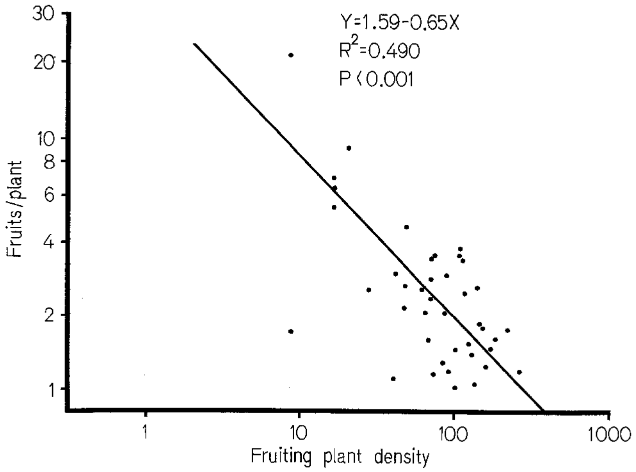

Fig. 6. Regression of log fruits per plant versus log fruiting plant density (plants per dm^2) in 41 undisturbed plots

Table 3. Means for undisturbed plots at two times. Density is equal to the number of plants per dm^2

	Time of flowering			Time of fruiting			
	Soil depth	Density	Flowers/ Plant	Soil depth	Density	Fruits/ Plant	Seeds/ Fruit
Mean	15.22 mm	127.9	2.20	17.99 mm	92.17	2.09	7.35
S.E.	1.08	15.13	0.05	1.53	8.72	0.04	1.03
N	41	41	5,243	41	41	3,789	410

Discussion

Earlier work on this species by Sharitz and McCormick (1973), conducted in a laboratory situation, demonstrated density and fecundity were both affected by soil depth and moisture availability. The effects of density on fecundity were not explicitly discussed, but recalculation of their data showed that individuals at initial densities of 500 plants per dm^2 produced twice as many seeds as individuals at initial densities of 1,000 plants per dm^2 while mortality was the same at both densities. Our experiment, conducted in natural field conditions, confirmed that reproduction in this species is density-dependent; additionally, significant differences among sites demonstrate that physical factors are also strong determinants of reproduction.

The reproductive responses of *Diamorpha smallii* to varying densities in a natural population are similar to the density responses reported for many plant species raised in artificial environments (Harper 1977). The mean flower number per plant decreases with increasing density. The trend of increased skewness with density is the same trend reported by Obeid et al. (1967) for *Linum usitatissimum*, grown at three densities. They found that the number of capsules per plant for the two lower densities was lognormally distributed; however, at the highest density, this variable was significantly skewed from a lognormal distribution. This supported Koyama and Kira's (1956) argument that a lognormal distribution is expected at low densities because of the exponential nature of plant growth, whereas at high densities, increased variation in individual growth rates under competition leads to increasingly skewed distributions. The distributions of the number of flowers per plant in our study were significantly skewed from a lognormal distribution at all densities.

Other reproductive characters have been reported to respond to density in other species, but the relation between the response of the character and density varies from character to character (Harper 1977; Krebs 1971; Obeid et al. 1967). In our study, the number of flowers and fruits per plant showed a strong response to density whereas the number of seeds per fruit showed only a slight, non-significant response to density. Obeid et al (1967) also reported that the number of fruits per plant was influenced to a greater extent by density than was the number of seeds per fruit.

The relationship of plant response to density is often expressed as a regression equation. The relationship between log total plant weight versus log plant density has been shown to have a slope of $-^3/_2$ for many species in a variety of conditions (Yoda et al. 1963; White and Harper 1970) and is known as the law of self-thinning. The regression of log number of flowers (fruits) per plant on log density had a slope of -0.73 (-0.65) for natural plots of *Diamorpha smallii.* We would not expect a slope of $-^3/_2$ since it is unlikely that the number of flowers (fruits) per plant is linearly related to total plant weight.

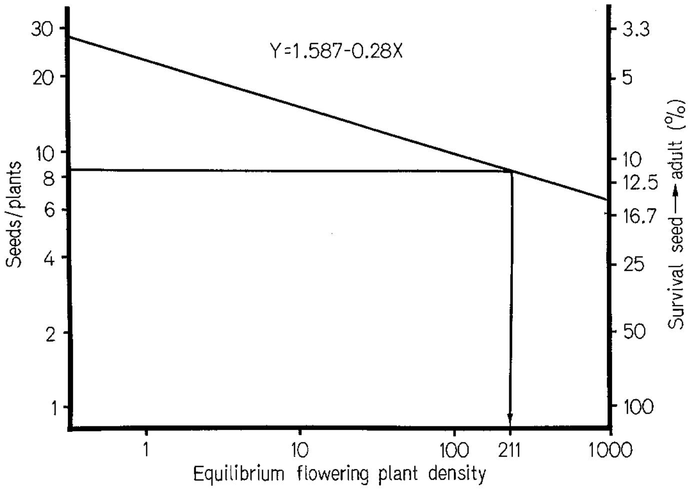

Fig. 7. Population model. Relationship between replacement seed production rate (*left axis*) for a given seed to adult survivorship (*right axis*) and the equilibrium flowering plant density. An equilibrium density of 211 plants per dm^2 is predicted for 11.4% survival from seed to adult

The differences between the regression slopes for the experimental and natural plots may result from the fact that, in the experimental plots, seedlings originally experienced high densities prior to our experimental thinning. In the natural plots however, seedlings at low densities were responding to those densities prior to our experimental manipulations. Therefore, plants in areas of naturally low density had a "head start" over those in the low density experimental treatments. Site differences may also contribute to differences in the slopes.

Watkinson and Harper (1978) were the first to demonstrate an effect of density on fecundity using experimental approaches in a natural population of a grass, *Vulpia fasciculata.* By thinning some extant stands and by adding seed to others, they obtained a wide range of densities. Mortality appeared to be density-independent, but fecundity was strongly negatively density-dependent. Based on the results of their experiment, they developed a population model from which they could predict the equilibrium population densities, depending on the survival rate.

Using the regression equations obtained in this study we constructed a similar population model in order to estimate equilibrium densities and to better compare *Diamorpha smallii* with *Vulpia fasciculata.* The equilibrium is considered to be that density at which each individual plant leaves, on the average, one reproductive offspring. The relationship between log seed production per plant and log adult plant density is described by the equation $Y=1.587-0.28X$. This relationship is obtained by adding the equation ($Y=1.027-0.08X$) for the relationship of log seeds per fruit, from the undisturbed plots to the equation for the relationship of log flower per plant, from the experimental plots ($Y = 0.56 - 0.20X$). Sharitz and McCormick (1973) reported that 1.4% of the seeds produced survived to maturity, therefore an individual must produce on the average 71 seeds to replace itself. Setting Y equal to the log of 71 (1.85), and solving or X, the predicted equilibrium density is approximately 0.12 plants per dm^2 (Fig. 7), a density well below densities observed at the Overton outcrop. However, the 1.4% survival rate reported by Sharitz and McCormick (1973) was based on observations from an outcrop in Georgia. Data from the experimental controls in our study suggest that survival may be considerably higher at Overton, approximately 11.6%. This value was estimated by calculating the expected seed output from the number of flowering plants present in the control plots at the end of the study. The number of the reproductive plants divided by the seed production was defined as the percent survival. This estimate is dependent on the assumption that after seed dispersal there is no net gain or loss of seeds in the plot. Using the value of 11.6% survival, the predicted equilibrium density is 211 plants per dm^2, a density in the middle of the range of densities observed in the natural plots but higher than the mean density. The estimated mortality, 88.4%, includes both density-dependent and density-independent mortality. For the purpose of the model we have not attempted to separate the two components of mortality since mortality was only roughly estimated. Inclusion of density-dependent mortality in the model would result in a steeper slope if log density and mortality were linearly related. If the model were refined in this way, predictions of equilibrium population size would be made from estimates of density-independent mortality.

The equilibrium density predicted by the model for this population is above the threshold density at which plants cease to interact, as determined from the experimental field manipulations. Therefore, at equilibrium densities, the fecundity of *D. smallii* individual is constrained by its neighbors. Antonovics and Levin (1980) have introduced the concept of the "ecologically effective distance" which defines the limiting, or threshold, distance at which neighbor effects cease. For *Diamorpha smallii*, we suggest this distance is about 2 cm, since at greater spacings there is no significant difference among means of the log of the number of flowers per plant. In populations in which all plants are spaced at this distance or more (equal to a density of 25 plants per dm^2 or less), the plants do not reduce their neighbors' fecundity, and reproduction is density-independent. The leveling off of individual flower production with decreasing densities observed in experimental plots was not observed in natural plots. This indicates natural self-thinning (density-dependent mortality) does not lower densities beyond the point at which direct plant-plant interactions influencing fecundity become negligible. The distance between plants in the natural plots was always less than, on the average, the ecologically effective distance. The circular area around each plant having the ecologically effective distance at its radius was defined as the "control area" (Antonovics and Levin 1980). In this study the overall mean density was 128 plants per dm^2, and with an ecologically effective distance of 2 cm, there are, on the average, 16 adult plants within the control area around a single individual. In contrast, populations of *Vulpia fasiculata* (Watkinson and Harper 1978) had an average equilibrium density of 30 plants per dm^2, an ecologically effective distance (calculated from their data) of approximately 5 cm, and a control area of about 79 dm^2.

The values we have calculated for the equilibrium density and the ecologically effective distance should not be considered fixed, rather these values will vary depending on environmental fluctuations and the stage of the life cycle. The reason for determining these values lies not in assigning specific numbers but in establishing the concepts as operationally useful. Watkinson and Harper (1978) pointed out that concordance of the equilibrium density derived from their model with observed densities implies the verity of the assumptions of the model. This point is emphasized by our study of *Diamorpha smallii.* Although the two species have many differences in their basic biology, both species apparently have population sizes that are regulated by strongly density-dependent processes.

It is interesting to note that while *D. smallii* is a rare species, being restricted to relatively few sites in the southeastern United States, its numbers are clearly not limited by its reproductive capacity. Populations of other rare species (*Plantago cordata*, Meagher et al. 1976; Mertz 1971) have been shown to be generally limited in their capacities to recover following drastic reductions in numbers. Demographic characteristics of these species restrict the rate of increase. This is not the case for *D. smallii*, for which the specialized and restricted nature of its habitat and density-dependent processes within the habitat are the primary constraints on the species' abundance.

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