Reproductive biomass allocation in the dioecious perennial *Acanthosicyos horrida*

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In a Namib population of the dioecious perennial *Acanthosicyos horrida* Welw. ex Hook. f. in which fruit production is declining, we investigated the effects of plant gender, plant size, and subpopulation elevation (a proxy of water availability) on a plant's biomass allocation to sexual reproduction. While males invested more heavily in buds and flowers than females, females allocated 10 times more total biomass to reproduction per m³ of vegetation than males during our survey period. Also, smaller plants, particularly females, allocated more biomass to reproduction per m³ of vegetation than larger plants. This result suggests that plant fecundity per m³ of vegetation decreases with plant size. Aging of the population could thus be a cause of the reduced fruit production; however, we found that the population was skewed towards the smallest size classes. On the other hand, the fact that plants in the subpopulation at higher elevation above the river were only six percent as productive as plants in the subpopulation at low elevation suggests that the depth of the water table may be critical to fruit production and that a reduced groundwater level caused by nearby urbanisation could have long term effects on harvests.

Introduction

Acanthosicyos horrida Welw. ex Hook. (Cucurbitaceae) is a large, long lived, dioecious perennial that is endemic to the Namib Desert and that is threatened by human encroachment on its habitat. *Acanthosicyos horrida* is ecologically a keystone species (Klopatek and Stock 1994) and economically important for the Topnaar and Himba Herero peoples (Arnold *et al.* 1985), who have historically harvested the fruit for their own use and for use in the confectionery industry of South Africa (Arnold *et al.* 1985, Meeuse 1962, Sandelowsky 1977, 1990). The Topnaar community is concerned about a decline in fruit production in their populations (Breuninger 1997), a decline that affects harvest yields and threatens the long term survival of these plants. This decline in fruit set is thought to be caused by decreased groundwater levels brought about by human withdrawals of water in the growing Namibian resort towns of Walvis Bay and Swakopmund (Breuninger 1997, Klopatek 1992).

There are two principal ways that lowering groundwater could affect fruit production in *A. horrida*, a species that survives in the virtually rainless environment of the Namib Desert by tapping its long roots into the higher groundwater along the banks of ephemeral rivers (Herre 1974, Sandelowsky 1990). First, decreased access to water by plants could directly affect their ability to produce reproductive structures. In such conditions, females may be unable to

produce as many water-rich fruits, and males may produce fewer flowers, potentially causing pollen limitation and decreased fruit set in the population. Second, lowering groundwater levels could indirectly affect fruit production by limiting seedling recruitment because many seedlings may be unable to grow taproots at a sufficient rate to reach the deeper water table before the environment dries after an infrequent rain. If recruitment is limited, the average age, and probably size, of plants in the populations would be increasing. In many polycarpic plant species, once a plant reaches the size required for reproductive maturity, reproductive effort first increases for many years and then decreases in later years (Hegazy 1992, Silvertown 1982). Thus, the aging of the *A. horrida* populations due to poor seedling recruitment could potentially be a cause of the low harvests the Topnaar are experiencing.

To examine these possibilities, we measured plant size, plant location within the population, and total allocation to sexual reproduction for every plant in a Namibian population of *A. horrida*. We then determined how total allocation to sexual reproduction per cubic meter of vegetation was affected by (1) elevation of the plant above the river, as an estimate of relative groundwater depth, to determine if plants in an area with lower groundwater allocated fewer resources to reproduction than plants in an area with higher

groundwater, and (2) plant size to determine if smaller plants allocated more resources to reproduction than larger plants. We also recorded the gender of each plant in the population, and we used gender as a third factor in our analyses because the sexes vary widely in allocation to sexual reproduction in dioecious plants (Ågren 1988, Armstrong and Irvine 1989, Cipollini and Stiles 1991).

Methods

Study system

Acanthosicyos horrida, commonly known as !nara, is a leafless, spiny cucurbit unique to the sand dune habitat of the Namib Desert (Herre 1974). This species has a spreading habit, and plants cover sand mounds up to 1500 m^2 (Klopatek and Stock 1994). Sand mounds are formed by windblown sand accumulating around roots at the base of plants (Klopatek and Stock 1994), and the sand mound of each plant is proportional to the size of its root system. Sand mounds usually are a great enough distance from one another (an average of 114m between mounds in our study population) that distinguishing genetically distinct individuals is fairly easy.

The reproductive season for this dioecious perennial species is virtually yearlong. Males produce flowers all year, with the greatest number of flowers produced each day between July and April (Klopatek and Stock 1994). Females produce flowers for a period of over nine months from August to April (Klopatek and Stock 1994). We chose to make a thorough survey of a population four months into the fruiting season but before harvest began. We feel that such a survey is a good estimate of the state of the population during the critical period of harvest. It was impossible to monitor the population for the entire reproductive period because *A. horrida* fruits are harvested by humans from January through March during which time access to the plants is difficult and information on which plants have been harvested is unattainable.

In November and December 1997, we examined a population of 31 plants of *A. horrida* along the Kuiseb River in the Namib Desert of central Namibia. Fruits from this population of 13 male and 9 female reproductively active plants are harvested each year by the local Topnaar people who reside in the area just south of the town of Walvis Bay and who gave us permission to work with their plants. The Kuiseb River is an ephemeral river that drains into the town of Walvis Bay.

Determining a plant's relative elevation

The Namibian population of *A. horrida* that we examined spanned a section of the Kuiseb River approximately three kilometers long. Nearly half of the population was several meters in elevation above the riverbed while the remainder of the population sat on a bluff tens of meters in elevation above the Kuiseb. We observed no differences in the phenological stages of the two subpopulations. Using topographic maps of the population, plants were placed either in the category of 'low' elevation relative to the river or 'high' elevation relative to the river. It should be noted that all

plants 'high' above the river are generally closer to one another than to those 'low' in elevation relative to the river and thus other environmental factors, besides the increased depth of the groundwater with elevation gain away from the river, may also be important in this category.

Determining a plant's size

Because *A. horrida* plants have a spreading habitat and plants vary in the density of vegetation within the total area occupied by an individual, we used total vegetative volume as our estimate of plant size. We estimated total plant volume by multiplying plant area by average plant height and percent cover. To estimate the area of each ovoid plant, we measured its length and width and used the measurements to calculate area (area = p [0.25][length x width]). Percent cover was estimated by viewing the plant from above and determining in what fraction of the mound non-vegetated sand was visible. We measured plant height at three random locations in each plant.

Determining a plant's biomass allocation to sexual reproduction

We multiplied the average biomass of each type of reproductive structure by the density of the structure to estimate the biomass per cubic meter of vegetation that each plant allocated to each type of reproductive structure. We then calculated total allocation to sexual reproduction per cubic meter of vegetation by adding the biomass per cubic meter of vegetation for each type of reproductive structure.

To determine the average biomass (average dry mass) of each type of reproductive structure, we collected a random sample of each type of reproductive structure from male and female plants. We collected samples of reproductive structures from males and females separately because there was an obvious size differences between buds and flowers in the two sexes. We used biomass as our allocation currency because studies have found that biomass gives a comparable response to other currencies (such as N or P) when comparing reproductive allocation between sexes (Ågren 1988, Wallace and Rundel 1979). We collected ten flowers from each of seven male plants and three female plants. We dissected each male flower into perianth and stamen and each female flower into perianth, style, and ovary. Male flowers had no relictual ovaries. Five living and five aborted buds were taken from each of four female and seven male plants. For these flowers and buds, all parts were weighed, dried, and then re-weighed. Dry living and aborted buds do not differ in mass and thus are not differentiated. To determine the biomass of fruits 70mm and under, we used three size classes based on fruit diameter (class 1: 25–40mm, class 2: 40–55mm, class 3: 55–70mm). For fruits over 70mm, the diameter was recorded to the nearest mm. Diameter is a good parameter for estimating fruit volume in cucurbits (Fuller and Leopold 1975, Sinnott 1945). Five living fruits from each size class were weighed fresh. Because we did not have the facilities for drying these fruits, we used dry aborted fruits as a proxy for the dry mass of fresh fruits. For each size class we calculated the ratio of dry aborted fruits

to fresh fruits. In case the conversion factors (size class 1 dry/fresh weight = 0.46, size class 2 dry/fresh weight = 0.37, and size class 3 dry/fresh weight = 0.33) overestimated the biomass of the larger fruits, we recalculated fruit biomasses using the smaller 0.16 conversion factor found by Arnold *et al.* (1985) and Klopatek and Stock (1994) for *A. horrida* fruit pulp alone (without the less water-rich seeds), and we found no qualitative differences in our results.

To estimate densities of reproductive structures on plants, we counted the number of sexually reproductive structures on each sexually reproductive plant. Since many plants had thousands of flower buds, for plants with more than 200 buds, we selected a random subsection of each plant and counted the number of buds per open flower to determine a ratio of buds to flowers that we multiplied by the number of flowers to estimate the total number of buds per plant. We tested this approach and determined that the bud to flower ratio was fairly constant throughout a single plant. We also counted the number of aborted buds and flowers on each plant. On female plants, we counted the number of fruits (mature and immature) and recorded the size class (described above) of each fruit based on fruit diameter. The counts of each type of reproductive structure on each plant were divided by the volume of the plant to obtain the number of structures per cubic meter of vegetation.

Statistical analyses

A t-test (SAS Institute 1996) was used to determine if non-reproductive plants differed in vegetative volume from reproductive plants. Three-way ANOVAs (SAS Institute 1996) were used to determine if the biomass of all reproductive structures as well as the biomass of each reproductive structure per cubic meter of vegetation was significantly affected by plant gender, plant size, or distance from the river. Plant size ('small' or 'large') and elevation above the river ('high' or 'low') were categorical variables because the data were bimodally distributed. Also, nested ANOVAs (SAS Institute 1996) were used with plant (nested in gender) and gender as the main effects to analyse differences in total biomass between individual reproductive structures that were common to both sexes.

Results and Discussion

Population size distribution

The size distribution of this population of 31 *A. horrida* plants was heavily weighed towards the smallest size classes. While the mean size of plants was 32.64 ± 11.73 (SE) cubic meters, the median was only 3.62 cubic meters. Also, the nine non-reproductive plants were significantly ($p < 0.0084$) smaller in vegetative volume (= 1.26 ± 8.80 (SE) m³) than the 22 sexually reproductive plants (= 31.06 ± 5.76 (SE) m³). This result suggests that non-reproductive plants are immature plants, and thus a large fraction of this population is relatively small and immature.

Factors affecting total biomass allocation to sexual reproduction

We found that plant gender, plant vegetative volume, elevation of the subpopulation in which the plant grew, and all the interactions between these factors significantly affected the total level of allocation to sexual reproduction per cubic meter of vegetation (Table 1). Many of these factors also significantly affected the allocation to individual reproductive structures.

The biomass of total reproductive structures per cubic meter of vegetation was significantly greater in females (= 73.73 ± 12.26 [SE]g) than in males (= 7.30 ± 11.27 [SE]g). The difference in total reproductive biomass allocation between the sexes was caused by the high allocation of females to fruits (= 67.64 ± 54.16 [SE]g). Males in fact allocated significantly more biomass per cubic meter of vegetation to buds and flowers (= 6.04 ± 1.38 [SE]g to buds and =0.86 ± 0.16 [SE]g to flowers) than females (= 0.12 ± 1.51 [SE]g to buds and = 0.08 ± 0.18 [SE]g to flowers). Males allocated more to buds and flowers in *A. horrida* because although female buds were 1.88 times the dry mass of male buds, and female flowers were 3.31 times the dry mass of male flowers (Table 2), males produced 42.98 times as many buds and 12.55 times as many flowers per cubic meter of vegetation as females. The pattern of males allocating more to flowers and females allocating more to reproduction overall corresponds to what researchers have found in many dioecious and subdioecious species (Ågren 1988,

Table 1: Three-way ANOVA results (P-values) on the effects of plant gender (male vs female), plant size (small vs large), and elevation (high vs low) of the plant above the river on the biomass (g dry mass) of sexual reproduction structures per cubic meter of vegetation of 22 *Acanthosicyos horrida* plants

	df	Total of Reproductive Structures	Buds	Aborted Buds	Flowers	Fruits	Aborted Fruits
Gender	1	0.0013**	0.0119*	0.7272	0.0060**		
Size	1	0.0017**	0.4497	0.6501	0.9748	0.0267*	0.0396*
Elevation	1	0.0007***	0.0313*	0.9572	0.0050**	0.0229*	0.4835
Gender x Size	1	0.0026**	0.4004	0.8228	0.6192		
Gender x Elevation	1	0.0025**	0.0369*	0.3795	0.0145*		
Size x Elevation	1	0.0040**	0.5003	0.7697	0.6965	0.0399*	0.4835
G x S x E	1	0.0057**	0.4323	0.5560	0.3278		

Note: *** for p ,0.001, ** for p ,0.01, and * for p < 0.05

Table 2: Mean (± SE) dry masses (g) of individual reproductive structures in male and female *Acanthosicyos horrida* plants. Nested ANOVAs were used to determine if gender and plant (nested in gender) significantly affected the biomass of reproductive structures comman to both sexes, and p-values are reported for the effects of gender on biomasses of these structures. Plant (nested in gender) was a significant effect in biomass of flowers and perianths, but not buds

Structure	Males	Females	p
Bud	0.08 ± 0.01	0.15 ± 0.01	0.0002***
Whole flower	0.13 ± 0.01	0.43 ± 0.01	0.0001***
Perianth	0.09 ± 0.01	0.09 ± 0.01	0.2122
Ovary		0.20 ± 0.07	
Style		0.02 ± 0.01	
Stamen	0.04 ± 0.01		
Fruit		9.75 ± 0.58	
Aborted fruit		7.04 ± 2.26	

Note: *** for $p < 0.001$, ** for $p < 0.01$, and * for $p < 0.05$

Armstrong and Irvine 1989, Delph 1990, Popp and Reinartz 1988).

Small plants allocated significantly more biomass per cubic meter of vegetation to total reproduction (= 72.78 ± 12.12 [SE]g) than large plants (= 8.26 ± 11.41 [SE]g). In particular, as seen in the significant two-way interaction between plant gender and plant size, small female plants allocated significantly more to reproduction per cubic meter of vegetation than did large female plants or male plants of any size. This decrease in allocation by size in females was due primarily to large plants producing many fewer grams of fruits per cubic meter of vegetation (= 9.86 ± 23.45 [SE] g/m³) than small plants (= 136.01 ± 33.17 [SE] g/m³). However, large plants produced significantly more aborted fruits (= 0.74 ± 0.16 [SE]g) than small plants, which produced none. The fact that smaller plants allocated such high levels to reproduction suggests differences in survival probabilities between large and small plants. For instance, if small plants are more likely to die each year than large plants, small plants may allocate more resources to current reproduction rather than to vegetative growth to increase uncertain future reproduction. Alternatively, root growth rates (affecting nutrient and/or water uptake rates) may not keep up with aboveground vegetative growth rates in these plants, and thus larger plants are not able to allocate as large a percent of their aboveground vegetative mass to reproduction as small plants.

Plants in the subpopulation at a lower elevation allocated significantly more biomass per cubic meter to reproduction (= 76.35 ± 9.84 [SE]g) than plants on the bluff (= 4.68 ± 13.43 [SE]g). In particular, plants at the lower elevation produced significantly more buds, flowers, and fruits, but not more aborted buds or aborted fruits than plants at the higher elevation. Also, as seen in the significant two way interaction between plant gender and elevation of the plant, female plants at the lower elevation allocated more to reproduction than females on the bluff or males at either elevation. There was also a significant interaction between plant size and elevation above the river. Small plants close to the river allocated significantly more total biomass to reproduction, particularly fruit production in females, than small plants

high above the river or than large plants of any size. The three-way interaction between plant gender, plant size, and elevation above the river was also a significant effect. Elevation may be an important factor influencing levels of reproduction in *A. horrida* because it reflects access to water, a limiting resource in this desert environment.

Conclusion

Our study suggests that plant gender, plant size, and the subpopulation elevation (as a proxy for water availability) significantly affect the total allocation to sexual reproduction by *A. horrida* plants in this Namibian population. In particular, we found that plants in an area with deep groundwater only allocated 6 % of the biomass to sexually reproductive structures as plants in an area with much shallower groundwater. This result gives credence to the hypothesis that dropping groundwater levels may be directly responsible for declining fruit harvests by the local Topnaar people. Our results do not support the hypothesis that dropping groundwater levels are hampering seedling recruitment (at least to a measurable level) because we found that the size distribution was skewed towards the smallest size classes, not the largest. However, if recruitment does become a problem in the future, the harvest in the population will probably diminish because smaller plants had ten times the allocation to reproductive structure per cubic meter of vegetation as larger plants.

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