

## SELECTIVE FRUIT MATURATION AND SEEDLING PERFORMANCE IN *ACACIA CAVEN* (FABACEAE)

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The tight globose inflorescences of *Acacia caven* commonly initiate several fruits, but only one or a few reach maturity. In this study, we ask whether natural fruit maturation patterns in this species are related to either fruit, seed, or seedling performance. We compared fruit, seed, and seedling characteristics from inflorescences that initiated several fruits but self-thinned naturally to mature one fruit, with inflorescences in which we chose randomly one immature fruit and removed all the others at an early stage of development. Also, we considered two additional natural treatments: inflorescences that initiated and matured two or more pods and inflorescences that naturally initiated and matured one fruit only. Around half of the fruits aborted in both manipulated inflorescences and inflorescences that initiated one fruit only. Pod size, seed number, and individual seed mass did not differ significantly between hand- and self-thinned inflorescences. Seeds from hand-thinned inflorescences showed, however, a significant decline in germination rate. Seeds from the other two additional natural treatments showed intermediate germination percentages. In addition, seedlings derived from germinated seeds produced by artificially thinned inflorescences also tended to perform poorly based on six quantitative seedling traits and seedling survival. The results indicate that progeny from hand-thinned inflorescences are of lower quality than progeny from inflorescences that underwent natural fruit abortion, supporting the hypothesis that *A. caven* matures selectively those fruits that contain more vigorous seeds.

**Keywords:** Mimosoideae, fruit abortion, seed quality, seedling survival, maternal fitness.

### Introduction

In many plant species, the number of flowers produced is one to three orders of magnitude higher than the fruits that are initiated, and the number of immature fruits is one to two orders higher than the number of fruits that reach maturity. This “waste” of reproductive resources has long puzzled evolutionary biologists (Sutherland and Delph 1984; Sutherland 1986; Ehrlén 1991, 1993; Guitián 1993, 1994). Many hypotheses have been advanced to explain this apparently inefficient reproductive behavior. Among them is the proposition that an initial “excess” of flowers, while making no numerical contribution to fruit set, may indirectly increase female reproductive success by allowing selective maturation of fruits containing high-quality seeds (“wider choice mechanism”; Burd 1998). This wider choice mechanism would provide fitness benefits to the maternal plant in years of surplus flower production (Burd 1998).

Indeed, in many species, fruits that reach maturity represent a nonrandom sample of those that are initiated. Nonrandom maturation may relate to fruit position within the plant or inflorescence, time of anthesis, or quantity and quality of pollination. Usually, fruits that have a more direct access to resources, derived from early opening flowers, or develop from flowers that received large or high-quality pollen loads are

those that tend to mature (Lee 1988; Solomon 1988; Herrera 1991; Richardson and Stephenson 1992; Obeso 1993; Bertin 1995; Aizen and Searcy 1998; Torres and Galetto 1999). Few studies, however, have demonstrated the fitness consequences for the maternal plant of nonrandom patterns of either fruit or seed maturation (Stephenson and Winsor 1986; Winsor et al. 1987; Casper 1988; Rocha and Stephenson 1991).

In this study, we specifically ask whether natural fruit maturation patterns in *Acacia caven* (Fabaceae, Mimosoideae) are related to either fruit, seed, or seedling characteristics. Usually, the tight brushlike or globose inflorescences that characterize the genus *Acacia* initiate several fruits, but only one or a few reach maturity (Peralta et al. 1992; Tybirk 1993; Baranelli et al. 1995). Because developing fruits within inflorescences are nourished by one central vascular bundle (L. Galetto, unpublished data), these types of inflorescences could act as “competition arenas” that screen among initiated fruits based on their strength as resource sinks. To prove this wider choice mechanism, we compare seed and seedling characteristics from inflorescences of *A. caven* that mature fruits naturally and inflorescences that were manipulated to produce one randomly selected fruit. In the opinion of Burd (1998), the artificial adjustment of maternal resources (e.g., hand-removal of competitors), followed by examination of fruit and seed quality, is the best protocol to assess the fitness consequences of natural patterns of either fruit or seed abortion. High maturation rates under unlimited resource availability should produce no change or even a decline in average fruit quality, whereas heavy

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abortion under resource restriction should lead to improvement in the fruits that do survive. If there is selective fruit maturation in *A. caven* because of resource limitation, we expect that fruits matured naturally by the plant—particularly in inflorescences that initiated more than two fruits but matured only one—will produce offspring of better quality than maturing fruits that were selected by chance by hand-removal of competing developing fruits.

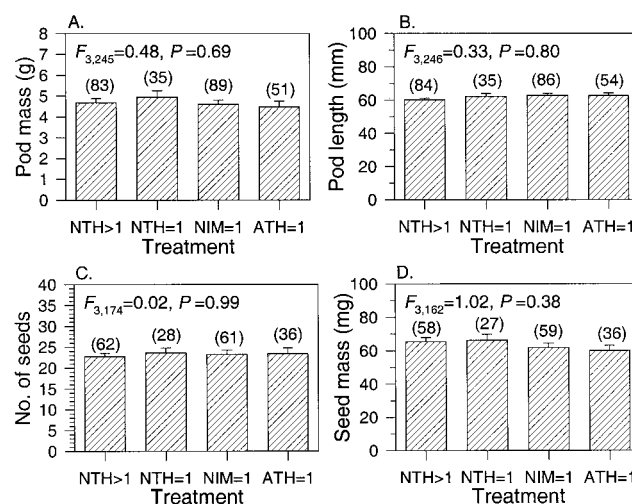
### Material and Methods

*Acacia caven* is a small tree characteristic of the arid and semiarid woodlands of southern South America. Plants flower early in spring and may produce several hundred small (1–1.5 cm), globose golden yellow inflorescences containing ca. 35 flowers. Each inflorescence bears a varying proportion of hermaphroditic and male flowers (Peralta et al. 1992; Baranelli et al. 1995). *Acacia caven* has been reported to be highly self-incompatible (Peralta et al. 1992). Inflorescences are visited mainly by beetles and several species of bees, wasps, and flies (Peralta et al. 1992; Baranelli et al. 1995). Successfully pollinated flowers develop into indehiscent pods containing up to 35 seeds. Of the high number of inflorescences produced, few mature fruits. An inflorescence may initiate several pods, but only one, or rarely two to five, reaches maturity (Baranelli et al. 1995 and data herein).

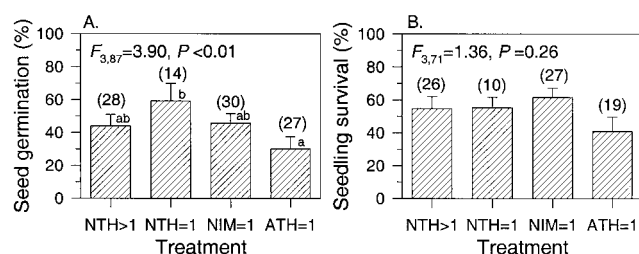
In August 1998, at the beginning of the flowering season, we marked 10 flowering trees of *A. caven* growing in a 10-ha remnant of Chaco Serrano Forest, near La Serranita, Córdoba Province, Argentina. The study site (31°44'S, 64°26'W) is located on the eastern slope of the Sierras Chicas, at 650 m above sea level. Just after the end of the flowering period, we selected haphazardly at least 50 inflorescences in each tree, including approximately equal numbers of inflorescences that initiated only one pod and inflorescences that initiated three or more pods. At that time, developing fruits were a few millimeters long (<15 mm). In half of the inflorescences that initiated three or more pods, we chose one pod randomly and removed all the others. Fruits from all marked inflorescences were harvested at maturity (ca. 4 mo later), weighed, and measured lengthwise. Seeds from each fruit were collected, counted, and weighed as a group on a microbalance to 0.001 mg. Individual seed mass was estimated by dividing total seed mass by the number of seeds contained in each fruit. A total of 15 seeds per pod (i.e., five seeds chosen randomly from the apical, central, and basal sections of each pod) were then placed on petri plates containing filter paper moistened with water, and the plates were placed in a germination chamber (2600–2900 lux; 12 h light/12 h darkness photoperiod; 22°C). We recorded a seed as germinated when we observed the emergence of the radicle. Seeds were checked at weekly intervals, and those that germinated were sowed individually in a 250-mL pot containing a 1:1 mixture of sand and a common compost soil. Pots were placed in a greenhouse at random positions under a 20°–30°C/10°–15°C day/night temperature regime. Two months after sowing, we harvested live seedlings and measured the following variables: seedling survival, shoot length, total number of leaves, and the leaf area of the four most basal leaves (scanned using the program Optimetrics software) in all live seedlings. Then seedlings were oven-dried at

30°C to a constant mass, and the total mass of roots, leaves, and shoot of each seedling was determined separately.

We were specifically interested in comparing fruits from inflorescences that initiated more than two fruits but matured one only (i.e., natural thinning [NTH] = 1) and fruits from inflorescences in which we randomly removed all developing fruits but one (i.e., artificial thinning [ATH] = 1). We considered, however, two additional natural treatments: inflorescences that initiated more than two fruits and matured two or more pods (NTH > 1) and inflorescences that naturally initiated and matured only one fruit (NIM = 1). These last two treatments might represent natural instances of limited selective fruit maturation. However, the lack of experimental contrasts for these two natural treatments allows for alternative interpretations (see “Discussion”). To avoid high levels of pseudoreplication (Hurlbert 1984), continuous traits of seeds and seedlings were averaged for each individual pod, and these values were considered as individual observations. Data on the different fruit, seed, and seedling variables (i.e., pod length; total pod mass; number of seeds per fruit; individual seed mass; percent seed germination; percent seedling survival; shoot length; number of leaves; leaf area; and root, leaf, and shoot biomass) were analyzed with two-way ANOVAs in which we considered treatment (1–4) and individual plant (1–10) as fixed and random factors, respectively. We did not include the interaction term in the final model because some treatments were not represented for some plants because of extensive fruit abortion in this species. This should provide a conservative test for the treatment effect because any variation accounting for the treatment × tree interaction would inflate the error term.



**Fig. 1** Fruit characteristics of *Acacia caven* according to different inflorescence treatments. Means + 1 SE of (A) pod mass, (B) pod length, (C) number of seeds per pod, and (D) individual seed mass. NTH > 1, inflorescences that initiated three pods or more and mature naturally two or more pods; NTH = 1, inflorescences that initiated three pods or more but self-thinned and mature only one pod; NIM = 1, inflorescences that initiated and mature one pod only; and ATH = 1, inflorescences that initiated three or more pods but were hand-thinned to mature only one pod. *F* values associated with the treatment effect are reported in each graph (see “Material and Methods”). Values in parentheses represent sample sizes.



**Fig. 2** Seed and seedling characteristics of *Acacia caven* according to different inflorescence treatments. Means + 1 SE of (A) percent seed germination and (B) percent seedling survival after 1 mo of germination. Notation as in fig. 1. For variables showing an overall treatment effect, means that share the same lowercase letter do not differ significantly (Tukey-Kramer test,  $P < 0.05$ ).

Given this unbalanced design that included empty cells, we considered the Type IV sums of squares (Shaw and Mitchell-Olds 1993). Raw data conformed to the assumptions of normality and homoscedasticity of ANOVA. We used MANOVA to determine significant differences in overall seedling performance among treatments. We considered the six seedling traits plus seedling survival as dependent variables. For each pod, we also computed a cumulative index of fitness by multiplying percent germination, percent seedling survival, and final seedling biomass. For this index, each of these three variables were standardized according to  $(X - \bar{X})/SD$  to give them equal weight (Zar 1984). We used ANOVA to determine significant differences in the cumulative index of fitness among treatments.

## Results

The number of developing fruits aborted by the inflorescences initiating one pod only (i.e., NIM = 1) and the inflorescences that initiated many pods but were hand-thinned to one developing pod (i.e., ATH = 1) was similar (55.9% and 56.8%, respectively). However, we recorded comparatively lower fruit abortion in inflorescences that initiated three or more pods (9.2%) (treatments NTH > 1 and NTH = 1 together). Nevertheless, 29.4% of these inflorescences matured one fruit only, indicating that extensive fruit abortion occur commonly in this species.

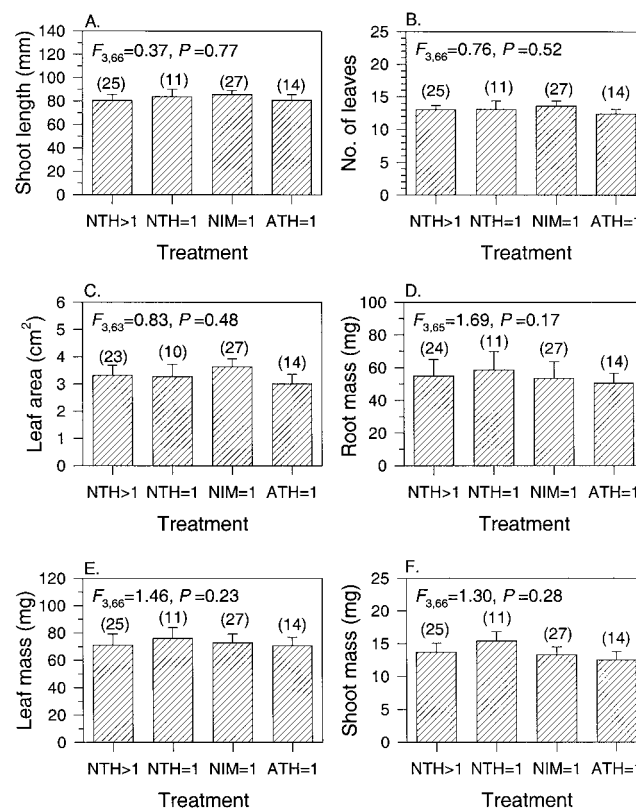
We did not find significant treatment effects for any of the fruit traits analyzed (fig. 1). In particular, there were no differences between treatments in either the size of the mature pods or the number or the mass of the seeds contained in those pods (fig. 1). However, seed germination was strongly affected by treatment. Inflorescences that initiated several pods but self-thinned to only one produced seeds with ca. 60% germination success, while germination in hand-thinned inflorescences was only half that value (fig. 2A). Seeds from the other two natural treatments (i.e., NTH > 1 and NIM = 1) showed intermediate germination percentages. Likewise, seedlings derived from germinated seeds produced by artificially thinned inflorescences showed the lowest survival (ca. 40%), although differences among treatments were not statistically significant (fig. 2B). In contrast, we found significant differences in pod mass ( $F_{9,245} = 2.38$ ,  $P < 0.05$ ), pod length ( $F_{9,246} = 9.40$ ,  $P <$

0.0001), number of seeds ( $F_{9,174} = 2.04$ ,  $P < 0.05$ ), individual seed mass ( $F_{9,167} = 3.81$ ,  $P < 0.0005$ ), and seed germination ( $F_{8,87} = 2.04$ ,  $P < 0.05$ ) among individual maternal plants, while seedling survival was marginally significant ( $F_{7,71} = 1.90$ ,  $P = 0.08$ ).

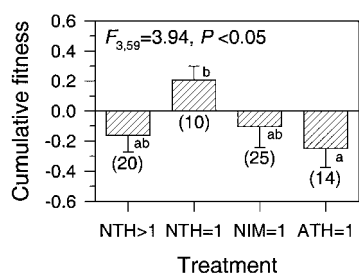
Quantitative characteristics of seedlings did not differ statistically among treatments (fig. 3). The performance of seedlings from hand-thinned inflorescences, however, tended to be the lowest for four of the six traits analyzed (fig. 3). In a MANOVA analysis that considered the six quantitative seedling traits plus seedling survival, we found significant differences in seedling performance among treatments (Wilks's  $\lambda = 0.41$ ,  $F_{21,45} = 2.14$ ,  $P < 0.01$ ).

The cumulative index of fitness showed significant differences among treatments. Seeds from inflorescences that initiated several pods but self-thinned to one only (NTH = 1) showed the highest average vigor, whereas seeds from the hand-thinned inflorescences (ATH = 1) showed the lowest vigor (fig. 4). Seeds from the other two natural treatments (i.e., NTH > 1 and NIM = 1) showed intermediate vigor values. The significance of treatment effect, evidenced by the MANOVA analysis, could be attributed mostly to the decline in seedling performance from hand-thinned inflorescences.

Like fruit and seed traits, we also found significant variation among trees for all the six quantitative seedling traits analyzed



**Fig. 3** Seedling characteristics of *Acacia caven* according to different inflorescence treatments. Means + 1 SE of seedling characteristics. (A) Shoot length, (B) number of leaves, (C) total leaf area of the four most basal leaves, (D) root mass, (E) total leaf mass, and (F) shoot mass. Notation as in fig. 1.



**Fig. 4** Cumulative index of fitness associated with seedlings from different treatments (see “Material and Methods”). Values are means  $\pm$  1 SE. Notation as in fig. 1. Means that share the same lowercase letter do not differ significantly (Tukey-Kramer test,  $P < 0.05$ ).

(shoot length:  $F_{7,66} = 8.06$ ,  $P < 0.0001$ ; number of leaves:  $F_{7,66} = 6.99$ ,  $P < 0.0001$ ; leaf area:  $F_{7,63} = 6.05$ ,  $P < 0.0001$ ; root mass:  $F_{7,65} = 6.03$ ,  $P < 0.0001$ ; leaf mass:  $F_{7,66} = 6.16$ ,  $P < 0.0001$ ; and shoot mass:  $F_{7,66} = 6.16$ ,  $P < 0.0001$ ).

### Discussion

The results of this study indicate that seeds from naturally and hand-thinned inflorescences differed the most in the percentage of seed germination (fig. 2). Moreover, seedlings from self-thinned inflorescences exhibited the highest cumulative fitness (fig. 4). Consequently, progeny from hand-thinned inflorescences was of lower quality than progeny from inflorescences that underwent natural fruit abortion, which supported the hypothesis that selective fruit maturation occurs in *Acacia caven*.

The main findings of this study parallel those reported for *Lotus corniculatus* (Stephenson and Winsor 1986; Stephenson et al. 1988). Self-thinned inflorescences of *L. corniculatus* produced seeds with higher germination success, as well as more vigorous seedlings, than hand-thinned inflorescences. In contrast to our study, these authors (Stephenson and Winsor 1986; Stephenson et al. 1988) also found that pods from self-thinned inflorescences contained fewer seeds than pods from hand-thinned inflorescences. Differences in seed number between naturally and hand-thinned inflorescences might not be expected in *A. caven*, however, because the pollination unit in the genus *Acacia* is a polyad composed of 32 grains (Peralta et al. 1992; Baranelli et al. 1995). In general, one polyad is enough to fertilize all ovules in the ovary. Genetic similarity among pollen grains implies that seeds within a pod also will be of similar quality. Therefore, selective fruit abortion in *Acacia* might be occurring based not on differences in seed number as in *Lotus* but on the overall vigor of the developing fruit and the seeds it contains (Shaanker et al. 1988). However, it is interesting to note that in our study, we did not detect differences in either pod or seed size between naturally and hand-thinned inflorescences. Physiological compartmentalization and allocation of fixed amounts of resources to each fruit within an inflorescence might explain the lack of differences among treatments (Casper and Nieserbaum 1993). Nevertheless, the differences in the number of aborted fruits between inflorescences that initiated more than three fruits with those that matured only one fruit would indicate that multiple-pod

inflorescences may sequester relatively more resources acting as more powerful sinks.

In addition to naturally and hand-thinned inflorescences, we also assessed fruit and progeny characteristics from inflorescences that self-thinned to a more limited extent (i.e., inflorescences that initiated and matured only one fruit, and inflorescences that initiated and matured more than one fruit). We expected these inflorescences to produce progeny exhibiting similar performance as those of hand-thinned inflorescences because of more restricted opportunities for selective abortion. Seed germination and the cumulative index of fitness in these two treatments were intermediate between hand- and self-thinned inflorescences (figs. 2A, 4), which partially supports this expectation. However, we did not find a similar pattern for individual traits related to seedling performance (fig. 3). Because levels of fruit and whole infructescence abortion in *A. caven* are so high, opportunities exist for plants to abort selectively those flowers/inflorescences that received low-quality pollen. Thus, inflorescences with apparently fewer opportunities for selective fruit maturation could already have been the product of a selective mechanism operating at an among-inflorescence scale.

With the exception of seed germination and the progeny cumulative index of fitness, our results showed zero-to-slight effects of nonrandom patterns of fruit maturation on other traits related to progeny performance. This contrasts with strong intraspecific phenotypic variation for almost all the variables analyzed. Thus, our results imply a heterogeneous mating environment with plants that differ in their capacity of producing, and perhaps siring, offspring of different quality. However, our results indicate that plant fitness in *A. caven* might be affected only to a limited extent by selective fruit maturation. In general, heritability of characters related to plant fitness is low for noncultivated plants, indicating that the response to selection will be slow (Wulff 1995). Nevertheless, maternal genotype by environment interactions are common in the wild (Wulff 1995 and references therein), indicating that there is a potential for selection at specific combinations of maternal and progeny conditions in natural habitats. This site-specific selection cannot be revealed by growing seedlings under a common environment as we did. Therefore, future studies should consider the possibility that plants selectively mature offspring that perform better within the local environment surrounding a given maternal plant than elsewhere.

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