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Regular research paper

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## EFFECTS OF SEXUAL REPRODUCTION ON GROWTH AND VEGETATIVE PROPAGATION IN *ARUM MACULATUM* L. (ARACEAE): *IN-SITU* REMOVAL EXPERIMENTS

**ABSTRACT:** We investigated the effects of sexual reproduction on vegetative propagation and relative somatic cost in *Arum maculatum* L. (Araceae). Two groups were selected as control and experimental. The spadices of individuals in experimental group were removed to test the relationship between sexual reproduction and vegetative propagation.

Statistically significant differences were found between initial and final tuber weight in individuals with sexual organs removed. No significant differences were found between initial and final tuber weight in naturally reproducing (sexual organs not removed) individuals. However, the statistically significant differences were found between control and experimental group with respect to above-ground biomass but not in terms of below-ground biomass. The differences between two groups in terms of above-ground and below-ground nitrogen concentrations were also significant.

Relative somatic cost of sexual reproduction (RSC) was observed in above-ground parts, but not in below-ground parts in terms of biomass and in both above- and below-ground parts in terms of nitrogen concentration.

**KEY WORDS:** *Arum maculatum*, sexual reproduction, vegetative propagation, relative somatic cost, resource allocation.

### 1. INTRODUCTION

Plant sexual reproduction normally requires large amounts of resources. As a result of the costs associated with the development of reproductive structures, allocation to other functions, including vegetative reproduction, can be reduced. Hence, in plants capable of vegetative propagation, it has been found that larger allocation to sexually-related reproductive structures (fruits, seeds) caused reduced allocation to asexually-related regenerating structures, *i.e.*, tubers, rhizomes (Cruz and Moreno 2001). Thus trade-off between sexual reproduction and vegetative propagation may depend on environmental conditions, competitive dominance, life span, and genetic factors (Yufen and Dayong 2007).

Reproduction is expected to occur at a cost of survival, growth or future reproduction. Such costs are often referred to as reproductive trade-offs, because resources allocated to one beneficial trait may result in detriment to another. Trade-offs are generally detected as either correlated demographic responses among different reproductive classes or shifts in physiological allocation among life-history traits. Low levels of flowering and high levels

of dormancy further support that survival is paramount to these plants, while sexual reproduction is not. Because survival increases with size, growth and survival are considered in herbs to trade-off with sexual reproduction (Shefferson and Simms 2007).

Sexual reproduction is crucial for most plants, because it does not only provide genetic variation as a basis for adaptation to long-term environmental changes, but also promotes dispersal in time and space (Weppler and Stöcklin 2005). Several authors have pointed out an energy cost for both sexual reproduction and vegetative propagation (Snow and Whigham 1989, Méndez and Obeso 1993, Worley and Harder 1996).

Transporting of photosynthetic products from senesced leaves to storage tissues (*i.e.* lignified parts and below-ground organs) and reproductive structures is known as reproductive allocation (Obeso 2002). Allocation models are built to find the optimal proportions of resources allocated into different sinks (Kozłowski 2006). This process has a remarkable effect on plant life span (Weiner 1988). Saikkonen *et al.* (1998) found that reproductive allocation during sexual reproduction inhibits vegetative reproduction. Plants may vary in their allocation to sexual and clonal reproduction and an investment in clonal growth may imply a trade-off with sexual reproduction (Weppler and Stöcklin 2005).

Araceae in general and the subfamily Aroideae in particular present a great diversity of developmental features relating to floral biology and a unique system for studying the transition of different floral types along the same inflorescence. Members of the Araceae produce elaborate inflorescences that attract their insect pollinators with a wide range of scents and inflorescence design, including trap pollination systems (Diaz *et al.* 2006, Barabé *et al.* 2003). *A. maculatum* is

a member of Aroideae widespread in Northern Turkey under deciduous forest canopies and on damp places.

The objective of this study is: (a) to assess the expected relationship between vegetative and sexual reproduction by *in-situ* removal experiments; (b) to calculate reproductive somatic cost of *A. maculatum*.

## 2. STUDY AREA

*A. maculatum* specimens were collected from three different localities, namely site I (Hacı Osman Forest, 41°14'57"N; 36°31'31"E), site II (Yörükler, 41°31'26"N; 36°05'05"E) and site III (Kurupelit, 41°22'04"N; 36°11'14"E) distributed in North Turkey close to Black Sea coast. Hacı Osman Forest is a swamp forest, and Hacı Osman Forest and Yörükler village are at the sea level, while Kurupelit is 200 m from the sea level. *A. maculatum* individuals usually occurred with *Ruscus aculeatus* var. *aculeatus* individuals. In site I, *A. maculatum* individuals occurred under a swamp forest dominated by a close canopy of *Fraxinus angustifolia* Vahl. subsp. *oxycarpa* (Bieb. ex Willd.) Franco et Rocha Afonso, while in site II and site III *A. maculatum* individuals occurred under a mixed forest of *Quercus cerris* L. var. *cerris* and *Q. petraea* (Mattuschka) Liebl. subsp. *iberica* (Steven ex Bieb.) Krasn. Population density of *A. maculatum* was found to be 185, 129, 101 in site I, II and III, respectively, per 100 square meters.

Climatic characteristics of the study area are shown in Table 1. The area may be characterised as having a semi-humid Mediterranean climate (Daget 1977). Pluviometric quotient (Q) is 76.7. Mean annual relative humidity is 79% (Turkish Ministry of Agriculture 2002).

Vegetative growth period of the studied species starts in the middle of November by emerging young shoots. Flowering period ap-

Table 1. Climatic characteristics of the study area (Turkish Ministry of Agriculture 2002).

Site	Locality	Mean annual temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Mean annual precipitation (mm)
I	Hacı Osman Forest	13.8	37	-2	895
II	Yörükler	13.5	39	-1.5	672
III	Kurupelit	14.2	37	-7	668

pears between the first week of April and third half of May. Fruit ripening occurs between the third half of May and the second half of July.

### 3. METHODS

*A. maculatum* individuals were randomly selected and flagged during June 2001 in site I (n = 180) and April 2002 in site II (n = 59) and site III (n = 29). An experimental group was set up in each of the three localities to test the effects of the removal of spadices on growth and vegetative propagation. The spadices of 33 (2002) and 50 (2003) individuals in site I and of 24 (2003) and 14 (2003) individuals in site II and site III were removed when the spadix emerged.

The tubers were dug out with the help of a soil auger during September 2002 and put into polyethylene bags and brought into the laboratory. The soil particles and plant debris were removed. Tubers were kept in room temperature to allow them drying until the constant weight. Tubers reached the constant weight in 15–18 days (the difference between two estimation of weight was less than 0.1 g).

The dried tubers were planted again in the same area. Then the plants were visited weekly from the beginning of the vegetative growth period. The date of the emergence of the first leaf, the number of leaves, date of the emergence of the spadix, date of flowering, fruit ripening and date of senescence were recorded. The dead leaves were brought to the laboratory and weighed after drying at 60°C. At the end of the growth period, the number of fruits of the individuals in the control group were counted and the fruits were collected, brought to laboratory and dried at 60°C.

During September 2003, tubers were dug out again and the number of daughter tubers, dry final tuber weight and daughter tuber weight were determined. Growth of tubers was estimated as the changes in mass between the onset and end of growth period.

To determine somatic cost of reproduction, biomass and total nitrogen amount were compared for above- and below-ground parts in reproductive and nonreproductive individuals. Relative somatic cost (RSC) was determined by using Tuomi's formula

$$RSC = (I_s - I_n) / I_n \quad (1)$$

where  $I_s$  is the biomass or total nitrogen content of plants with reproductive structures removed, while  $I_n$  is the biomass or total nitrogen of control plants (Kilinc *et al.* 2006).

For the estimation of total nitrogen amount above- and below-ground parts were dried at 60°C until constant weight, ground, and sieved. Nitrogen was determined by the micro Kjeldahl method with a Kjeltec Auto 1030 Analyser (Tecator, Sweden) after digesting the samples in concentrated  $H_2SO_4$  with a selenium catalyst (Kilinc *et al.* 2006).

PATH analysis was used to determine the effect of sexual reproduction on growth and vegetative propagation. PATH analysis was carried out by using TARIST software (Açıkgoz *et al.* 1994). PATH analysis is a generalization of multiple regression that allows to estimate the strength and sign of directional relationships for complicated causal schemes with multiple independent variables. In analysing of growth, final tuber weight was selected as dependent variable, while initial tuber weight, total leaf weight, weight of reproductive structures, daughter tuber weight and fruiting time were selected as independent variables. In analysing of vegetative propagation, daughter tuber weight was selected as dependent variable, while initial tuber weight, weight of reproductive parts, total leaf weight and fruiting time were selected as independent variables.

Additionally one-way ANOVA was used to test differences between initial and final biomass and nitrogen amount of individuals. The differences between manipulated and control individuals in terms of final tuber weight and nitrogen amount were also tested by using one-way ANOVA. In order to determine the relationship among tuber weight, leaf weight and the number of leaves regression analysis was used (SPSS 10.0 version, Anonymous 1999).

### 4. RESULTS

There was a difference between initial and final tuber weight in individuals with sexual organs removed (Table 2).

According to the results of PATH analysis, initial tuber weight and total leaf weight had positive effects on final tuber weight, weight of reproductive structures and daughter tu-

Table 2. Mean values of initial and final tuber weights ( $\text{g m}^{-2}$ ) of naturally reproducing (spadices not removed, control) and with sexual reproduction prevented (spadices removed, manipulated) individuals.

	Group	Mean Biomass ( $\text{g m}^{-2}$ )	<i>P</i>	Mean N concentrations (%)	<i>P</i>
Initial tuber weight	Control	19.34 ± 1.68	ns	0.92 ± 0.38	
	Manipulated	19.90 ± 1.40			
Final tuber weight	Control	21.59 ± 2.07	**	0.53 ± 0.10	**
	Manipulated	25.96 ± 2.17			

(\*\**P* < 0.01; ns: not significant)Table 3. The effects of independent variables on final tuber weight and daughter tuber weight (dependent variables) in *A. maculatum* populations in PATH analysis.

Independent variables	PATH coefficient
Dependent variable: Final tuber weight	
Initial tuber weight	0.34
Total leaf weight	0.41
Weight of reproductive structure	-0.03
Daughter tuber weight	-0.02
Fruiting time	-0.25
Dependent variable: Daughter tuber weight	
Initial tuber weight	0.19
Total leaf weight	0.34
Weight of reproductive structure	-0.20
Fruiting time	-0.25

Table 4. Above – and below-ground biomass (in g) and nitrogen concentrations (in %) of the naturally reproducing (spadices not removed, control) and with sexual reproduction prevented (spadices removed, manipulated) individuals. Statistical results are from one-way ANOVA.

		Biomass values			
		Mean ± SD	F-value	df	Probability
Above-ground	Control	1.70 ± 0.12	7.29	1	0.008 **
	Manipulated	1.30 ± 0.09			
Below-ground	Control	21.59 ± 2.07	0.11	1	0.746 ns
	Manipulated	25.96 ± 2.17			
		N concentrations			
Above-ground	Control	2.43 ± 0.41	36.01	1	0.000 **
	Manipulated	1.75 ± 0.32			
Below-ground	Control	0.92 ± 0.38	26.09	1	0.000 **
	Manipulated	0.53 ± 0.10			

(\*\**P* < 0.01; ns: not significant)

ber weight had almost negligible and negative effect, while fruiting time had negative effect on final tuber weight (Table 3). Initial tuber weight and total leaf weight had positive effects on daughter tuber weight, while weight of reproductive structures and fruiting time had negative effects on daughter tuber weight (Table 3).

Statistically significant differences were found between initial and final tuber weight in individuals with sexual organs removed ( $F = 5.46$ ;  $df = 1$ ;  $P = 0.020$ ). No significant dif-

ferences were found between initial and final tuber weight in naturally reproducing (sexual organs not removed) individuals ( $F = 0.70$ ;  $df = 1$ ;  $P = 0.401$ ). There were statistically significant differences between manipulated and the control plants with respect to above-ground biomass, whilst no significant differences were found in terms of below-ground biomass (Table 4). However, total nitrogen concentrations were higher in control plants as compared to individuals with spadices removed in both above- and below-ground

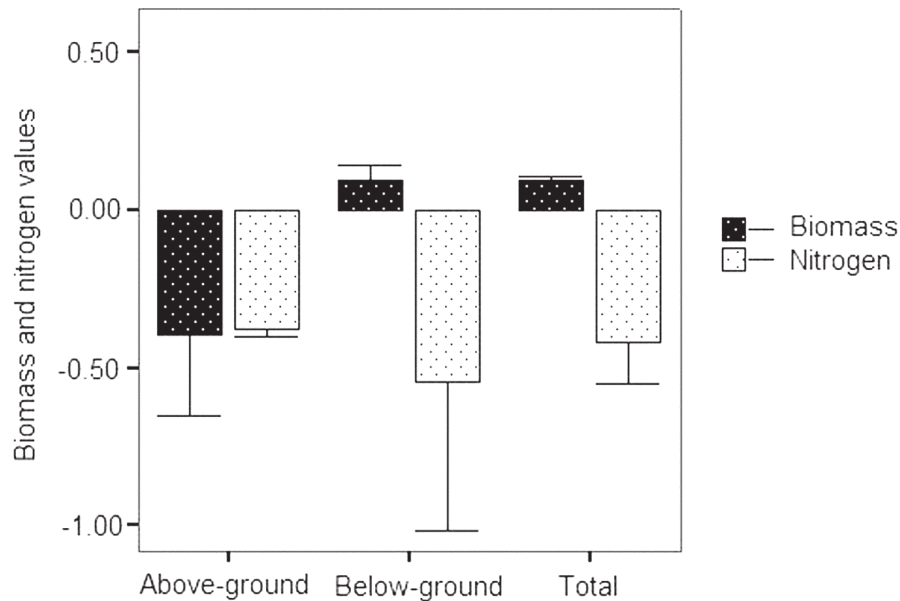


Fig. 1. Relative somatic cost of sexual reproduction (RSC) (formula 1) in terms of biomass and nitrogen content in *A. maculatum*. Negative values mean the cost.

parts; the differences were statistically significant (Table 4).

There was a significant correlation between tuber weight and leaf weight in both groups (control:  $r = 0.83$ ;  $F = 74.56$ ;  $df = 1$ ;  $P < 0.001$ , manipulated:  $r = 0.86$ ;  $F = 112.41$ ;  $df = 1$ ;  $P < 0.001$ ). Similarly, a significant correlation was also found between tuber weight and leaf number (control:  $r = 0.74$ ;  $F = 23.76$ ;  $df = 1$ ;  $P < 0.001$ , manipulated:  $r = 0.81$ ;  $F = 56.94$ ;  $df = 1$ ;  $P < 0.001$ ).

RSC was negative in above-ground parts, and positive in below-ground parts in terms of biomass. However, all RSC values were negative when nitrogen concentrations were used (Fig. 1).

## 5. DISCUSSION

As a result of *in situ* removal experiments, it was found that there was a clear difference between initial and final tuber weight in individuals with sexual organs removed. The switch between vegetative propagation and sexual reproduction shows, together with increased vegetative plant weight, that sexual reproduction brings about costs in

terms of growth in size and clonal propagation and shows a strong developmental link and less opportunity for flexible storage allocation (Jongejans 2004, Jongejans *et al.* 2006). Increased allocation to asexual reproduction can occur when allocation to sexual reproduction is reduced or absent. There is considerable evidence that plant populations differ with respect to the proportion of biomass allocated to reproductive structures in individual plants (Méndez and Obeso 1993, Méndez 1999, Coelho *et al.* 2005).

Photosynthetic products have been transferred to reproductive structures from below-ground structures. A significant increase was found in final tuber weight in individuals with sexual organs removed. However, such an increase was not found in sexually reproducing individuals. Two species (*Hypochaeris radicata* L. and *Succisa pratensis* Moench) also switched to increased rosette formation when flower buds were removed. If the relative somatic cost (RSC) has negative values, it can be concluded that reproduction creates a cost for plants (Jongejans 2004). Both above and below-ground structures have negative RSC values in terms

of nitrogen concentration. However, positive RSC values were found in below-ground structures in terms of biomass, whilst above-ground structures have negative values.

Under resource limitation, most clonal species show a marked increase in proportional allocation to vegetative propagation and a concomitant decrease in allocation to seed production. Verburg and During (1998) and Jongejans *et al.* (2006) found that sexual reproduction had demographic costs in the long run in long-lived perennials as a result of 3 years of continuous flower bud removal: inhibition of flower and seed production increased total biomass or rosette number. In the terms of adaptive biomass allocation, there is a trade-off between seed production and vegetative reproduction.

We found that total leaf weight and initial tuber weight have positive effects on final tuber weight in *A. maculatum* populations. Additionally, leaves were persistent after anthesis in *A. maculatum* populations, while leaves were dry at the beginning of growth period in naturally non-reproducing individuals or the individuals with their flowers removed. Méndez (1999) stated that the persistency of leaves after anthesis has a great impact in decreasing of reproductive cost.

No significant biomass increase occurred in tubers in naturally reproducing individuals, whilst a significant increase was found in individuals with sexual organs removed. Méndez (1999) found a similar result in *Arum italicum* individuals. In addition, it was found that fruiting time had negative effects on daughter tuber weight in *A. maculatum*. These results indicate that sexual reproduction has negative effects on vegetative propagation. The negative effects of sexual reproduction on vegetative propagation were especially strong in geophytes (Evenson 1983, Westley 1993, Horwitz and Schemske 2002).

Relative somatic cost (RSC) was negative regarding above-ground biomass, while it was positive with respect to below-ground biomass. However, RSC was negative in both above and below-ground parts when RSC was calculated as nitrogen amount. Negative values of RSC show that reproduction has a somatic cost. Méndez (1999) also found a somatic cost of reproduction despite low

sample size. Somatic cost of reproduction has been reported for other geophytic species (Snow and Whigham 1989, Primack and Hall 1990).

It has been reported that there was a competition between sexual reproduction and vegetative propagation for nutrients, and this competition may be reduced by the separation of sexual reproduction and vegetative propagation in time (Evans and Black 1993, Pantis 1993). Additionally vegetative reproduction is a very common alternative by which plants can contribute to next generations (Olejniczak 2003). The separation of sexual reproduction and vegetative propagation in time was observed in *A. italicum* Miller because at the onset of sexual reproduction the formation of daughter tubers decreased (Méndez 1999). In this study, it was also found that leaf weight and initial tuber weight had positive effects on daughter tuber weight. Méndez (1999) also found that initial tuber weight and total leaf weight have positive effects on daughter tuber weight in *A. italicum*. Sletvold (2002) reported that plant size has a positive effect on sexual reproduction.

Sexually reproducing individuals have higher nitrogen concentrations due to longer growth period as compared to individuals with reproductive structures removed. Nitrogen concentrations in leaves were higher than in tubers in both control plants and plants with spadices removed. Several authors reported that nitrogen and phosphorus were transferred from below-ground structures to leaves (Goldman and Willson 1986, Ashman 1994, Kutbay and Kılınç 2002). Resource allocation for vegetative growth, sexual reproduction and vegetative propagation significantly increased as dry mass of plant increased.

Summarizing, sexual reproduction has negative effects on vegetative propagation as well as plant growth. Statistically significant differences were found between initial and final tuber weight in individuals with sexual organs removed. Fruiting time and the weight of reproductive structures have negative effects on daughter tuber weight, while leaf weight and adult tuber weight have positive effects on daughter tuber weight. It may be concluded that leaf weight and number has a

pronounced effect on tuber weight and as a result plants with large above-ground part are also very effective on reproduction.

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