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Responses to Moisture Stress in Male and Female Plants of *Rumex acetosella* L. (Polygonaceae)

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Summary. Male and female plants of *Rumex acetosella* were grown on a moisture gradient to measure possible differences in the drought tolerance of the sexes. The growth of both sexes declined under water stress but males were significantly more drought tolerant. This could not be explained by greater water use efficiency in the male plants; measured rates of both photosynthesis and leaf conductance did not differ significantly between the sexes. Multiple discriminant analysis showed that the sexes differed at all moisture regimes in their overall patterns of biomass allocation. Males had proportionately greater investment in root and leaf tissue which could explain their growth advantage over females under water stress. Despite essentially equal water use efficiencies, on a *per plant* basis males, with more leaf and root biomass, could fix more carbon and more rapidly exploit the local water resource than females. Thus the pattern of biomass allocation rather than intrinsic physiological differences appears to explain the greater drought tolerance of male plants of *Rumex acetosella.*

Introduction

Diverse models have been put forward to explain the evolution of sex, a central and still unresolved problem in evolutionary theory (Bell 1982). Important among these is the suggestion by Ghiselin (1974) that sexuality is favored in crowded environments where sexual differentiation in resource utilization can confer an advantage on sexual morphs. If Ghiselin's model holds we should observe that the sexes of dioecious plant species occupy spatially or temporally distinct microhabitats. Such separation has beeen shown for a variety of woody and herbaceous species in habitats as diverse as cold desert and temperate forest (Davey and Gibson 1917; Mukerji 1936; Putwain and Harper 1972; Freeman et al. 1976; Grant and Mitton 1979; Wade et al. 1981; Cox 1981; and Fox and Harrison 1981). Both Wallace and Rundel (1979) and Hancock and Bringhurst (1980) failed, however, to find such separation of the sexes. Despite thorough reviews of secondary sex characteristics in plants (Dzhaparidze 1969; Lloyd and Webb 1977), we have no general understanding of the traits that can underlie habitat separations of the sexes in dioecious species (Bawa 1980).

A number of authors have described particular environmental contrasts in the habitats of male and female plants. Female *Populus tremuloides*, for example, predominates over males at lower but not at higher elevations in the Colorado Rocky Mountains (Grant and Mitton 1979). Mukerji (1936) and Wade et al. (1981) agree that male plants of the woodland herb *Mercurialis perennis* occur in sunnier microhabitats. Cox (1981), in contrast, was able only to relate the distribution of *Mercurialis* to soil pH and not to light regime. The microdistribution of the sexes in dioecious species has been most often related to water regime with females occupying moister habitats. This trend had been documented both for woody species like *Acer negundo, Myrica gale, Artemesia confertifolia* and *Ephedra viridis* and for herbaceous species like *Distichilis spicata, Thalictrum fendleri,* and *Hesperochloa kingii* (Davey and Gibson 1917; Freeman et al. 1976; Fox and Harrison 1981). Bawa and Opler (1977), however, were unable to show significant sexual differences in the distribution of four tropical trees over a riverine moisture gradient. These poorly defined and sometimes contradictory environmental correlates of microdistribution suggest the need for closer analysis of the traits underlying the response of the sexes to environmental gradients.

Materials and Methods

Red sorrel, *Rumex acetosella* L., is a low herbaceous perennial, native to Eurasia and widely naturalized on acid soils throughout North America (Gleason and Cronquist 1963). We collected and refrigerated rhizomes of each sex of *Rumex acetosella* in late August, 1979 from the grounds of the University of Michigan Biological Station near Pellston, Michigan. In November, 1979 the rhizomes were divided into segments approximately 3 cm long and placed in 5.7 cm peat pots which were filled with general purpose potting soil. Ninety segments of each sex were potted and allowed to grow before being transplanted to a greenhouse moisture gradient.

The moisture gradient was maintained by dripping distilled water into the lower end of inclined compartmentalized boxes (Pickett and Bazzaz 1976). All compartments were watered with 500 ml distilled H_2O if wilting occurred in the driest compartments. Figure 1 shows the gradient in soil moisture obtained. Auxiliary lighting maintained 99 μmol m^{-2} s^{-1} at the rosettes on a 16 h photoperiod; ambient greenhouse temperatures prevailed.

In mid-January, 1980 forty-eight of the largest sprouts (24 of each sex) were randomly transplanted into this moisture gradient. Four plants (two of each sex) were placed in each compart-

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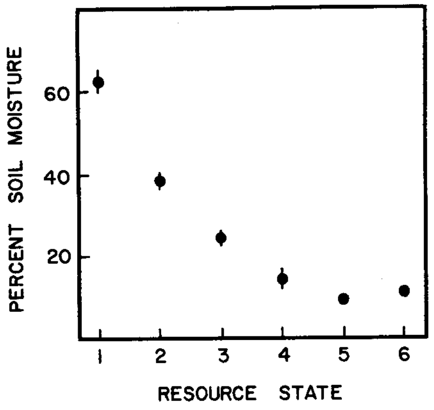

Fig. 1. Soil moisture as a percentage of soil dry weight along the gradient to a depth of 10 cm. Two dates (two replicates per day) are represented for each resource state. Bars indicate standard errors where larger than symbol

ment on two gradient boxes in random locations. The remaining plants were placed in rows in adjacent boxes to minimize edge effects among the experimental plants. Leaf counts were made to roughly estimate the initial biomass and developmental stage of each plant; males had 19.6 leaves compared to 15.5 leaves for females, a nearly significant difference ($P=0.06$).

During April 4–7, 1980, the photosynthetic and leaf conductance rates of each plant were measured in duplicate. Measurements were taken on one-half of the plants (one box randomly chosen) on each day. The order of measurement of individual plants was chosen in a stratified random manner – compartments were first randomly chosen and then individuals within compartments were chosen. Care was taken to select leaves of a constant age, estimated by their position in the rosette (younger leaves were more erect than older leaves). The condition of one plant necessitated using the lowest cauline leaves because all rosette leaves were senescent. Photosynthetic rates and leaf conductance were measured in close conjunction but on separate leaves. Because of wilting in the driest portions of the gradients, each compartment received 250 ml of distilled water following the completion of the first replicate measurement (April 5). All measurements were made between 1,100 h and 1,400 h: Eastern Standard Time.

Photosynthetic rates were measured by labelling leaves with radioactive $^{14}CO_2$ using a method slightly modified from Tieszen et al. (1974). Extra lighting was provided by PAR lamps (Sylvania) suspended approximately 1 m above each compartment during exposure to ensure the measured leaves were photosynthetically saturated (500 µmole m^{-2} s^{-1} PhAR or greater). Each compartment was illuminated at least 10 min before any measurements were made. Leaves were then exposed for 40 s to an air stream with 358 µl l^{-1} CO_2 with a specific activity of 5.20×10^{10} Bg $mmol^{-1}$ at a flow rate of 0.43 l m^{-1} through a clamp-on, 1 cm^3 minicuvette. A leaf disc was then immediately punched from the exposed tissue and frozen on dry ice to stop further metabolism. Samples were oven-dried at 75° C and combusted in an Intertechnique Oxymat catalytic furnace to recover $^{14}CO_2$ in a scintillation cocktail containing phenylethylamine. Radiometric assay was completed on a Beckman Model LS-200 liquid scintillation counter using the internal standards channels ratio method of quench correction (Wang et al. 1975). Leaf conductances were measured with a diffusive resistance meter (Licor model Li-60) following the precautions of Morrow and Slayter (1971).

Harvesting of plants occurred between April 10 and 14th, 1980. The plants were removed from the compartments, retaining as much of the underground biomass as possible. Soil was washed from the rhizomes and roots. The plants were partitioned into rhizomes plus roots, vegetative shoots including non-emergent shoots, living leaves and petioles, dead leaves and petioles, stem, and flowers or seeds. All material was placed in a drying oven at 70° C for at least 72 h before the dry weights of component tissues were measured.

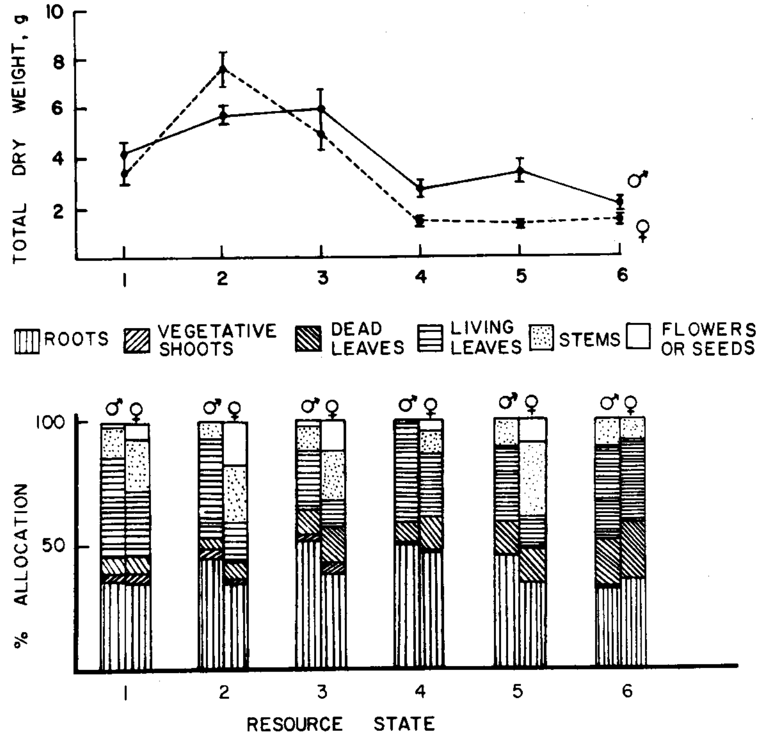

Fig. 2. Biomass accumulation of each sex in each resource state in grams dry weight (upper graph). Means are of four values, bars indicate standard errors. Percentage dry weight allocated to various parts for each sex in each resource state (lower graph)

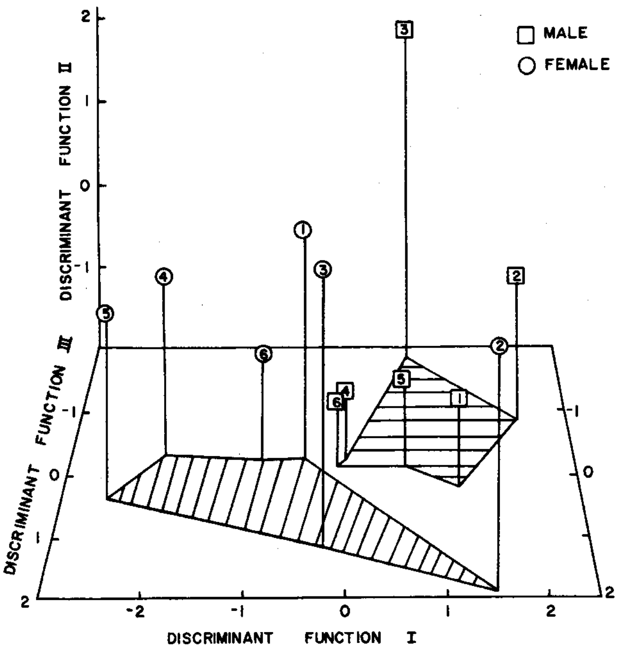

Fig. 3. Discriminant function scores for each sex in each resource state. Numbers within symbols indicate resource state as in Fig. 1. Standardized discriminant coefficients, variance accounted for, and significance test for each discriminant function appear in Table 1

All statistical analyses were performed using SAS (Barr et al. 1979) except for the multiple discriminant analysis which was performed using SPSS (Nie et al. 1975).

Results

Total Biomass and Allocational Responses

While the total biomass of male and female *R. acetosella* declines similarly with decreasing soil moisture (Fig. 2), analysis of variance indicated significant differences in total biomass between sexes ($P = 0.016$). In these analyses, a square-root transformation was used to normalize the data and provide equal group variances. Maximum biomass in either sex was achieved in resource states two and three. The female biomass peaks under wetter conditions than the male; and females grow larger than males under optimal moisture conditions. Males maintain significantly greater biomass under drought stress (compare means and error bars in states 4–6, Fig. 2).

Allocational differences between sexes were also apparent (Fig. 2). Due perhaps in part to the greenhouse photoperiod regime, more females flowered than males. Of seventeen individuals actually flowering at the time of harvest fourteen were female. This, and the fact that not all individuals of either sex had flowered, precludes an in depth consideration of differential reproductive effort (allocation to flowers or seeds) in response to the water gradient. A larger proportion of individuals had produced or begun to produce flowering stems (31 of 48) and in a more equitable ratio (15 males vs. 16 females) at the time of harvest. Concomitant with earlier flowering, females allocated greater biomass to the stem tissue that carried the inflorescence above the basal leaf rosette. Males allocated relatively more biomass to living leaves in all resource states and had equal or greater allocation to roots than females. Vegetative shoot production was small for both sexes and no significant differences appeared. There were also no consistent differences in the proportions of dead leaves.

Table 1. Discriminant function relativized coefficients, variance accounted for by each function, and associated tests of significance

	Discriminant functions		
	I	II	III
Relativized coefficients			
Roots and rhizomes	0.01	0.57	−0.13
Living leaves	1.00	−1.00	0.29
Dead leaves	0.43	−0.04	−0.26
Stem, flowers or seeds	0.29	0.21	1.00
Vegetative shoots	−0.24	0.94	−0.74
Cumulative variance accounted for (%)	41.9	67.9	89.1
Wilks' lambda	0.0361	0.1167	0.2786
Chi-square	127.91 (55)[a]	82.68 (40)	49.20 (27)
Probability	0.0001	0.0001	0.0056

[a] Degrees of freedom appear in parentheses

To more effectively assess between-sex contrasts these diverse allocational responses along the water gradient were analyzed from a multivariate perspective. Plant part dry weights for each sampled individual were used as variables in a multiple discriminant analysis (Morrison 1967; Gittins 1979) of the twelve (water by sex) groups. If the twelve groups fell into two major sets by sex then overall sex differences would be clearly indicated and general distinctions could more easily be summarized. All variables were either square root or log transformed to normalize the data and equalize group variances. Flower or seed weights were combined with stem-weights as total inflorescence tissue to reduce the number of zero values and allow the normalization of the data.

The multiple discriminant analysis of allocation did separate the two sexes into distinct, non-overlapping sets (Fig. 3). Three significant discriminant functions were obtained (Wilk's Lambda, $P < 0.05$) explaining 87.1% of the variance in the data (Table 1). Discriminant function I separated the groups predominantly by the contrast of rosette leaves and vegetative shoot weights (compare relativized coefficients in Table 1). Groups were separated along discriminant function II by the contrast between living rosette leaf weight and all over living tissue weights. Finally, discriminant function III largely separated groups by the balance of sexual versus vegetative reproductive tissues. In general females, regardless of moisture availability, had higher ratios of reproductive to leaf tissues (Fig. 3). Under optimal moisture conditions, females had higher ratios of sexual to vegetative reproductive tissues.

Physiological Responses

The sex differences in total biomass and allocation patterns are not reflected in measurements of photosynthetic rate and leaf conductance (Fig. 4). There is a strong correlation between the two parameters as one would expect. Analysis of variance for either variable indicated no significant differences between sexes (photosynthetic rates, $P = 0.689$; leaf conductance, $P = 0.094$). Plants growing under very dry or very wet conditions have low photosynthesis and low conductance regardless of sex. It is interesting that photosynthetic rates are high in resource state 4 dur-

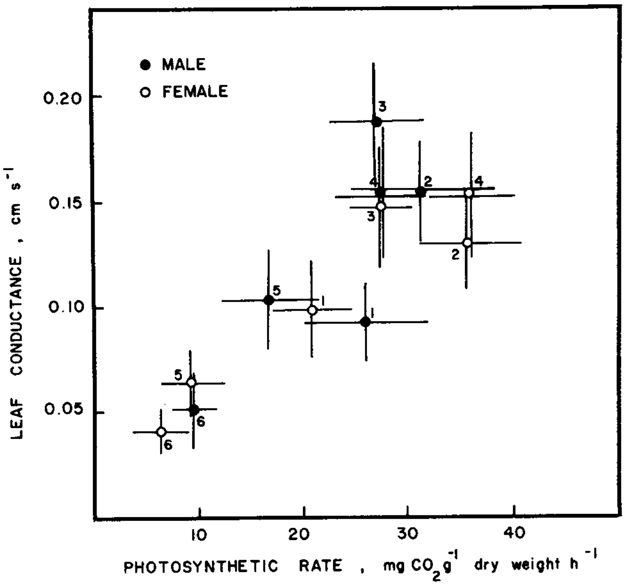

Fig. 4. Mean photosynthetic rates and mean leaf conductance for each sex in each resource state. Each point is a mean of eight values; bars indicate standard errors

ing the test period despite the low growth achieved under this moisture regime.

Discussion

In general, the growth responses of male and female *Rumex acetosella* over a gradient in soil moisture are similar to other temperate dioecious species (Davey and Gibson 1917; Lysova and Khiznyak 1975; Freeman et al. 1976; Fox and Harrison, 1981; but see also Wallace and Rundel 1979; Hancock and Bringhurst 1980). Males are more tolerant of xeric conditions than females. In contrast to the woody and herbaceous desert species studied by Freeman et al., however, the sexes have only slightly different soil moisture optima in *Rumex acetosella.*

It might be expected the physiological basis of these sexual differences in growth response would be readily apparent but this was not the case. A priori the more drought tolerant sex would be expected to have higher water use efficiency (higher photosynthesis and/or lower conductance) at low soil moisture. Wieland and Bazzaz (1975), for example, show that the more drought tolerant *Setaria faberii* has higher photosynthetic and lower transpiration rates than *Polygonum pensylvanicum.* Since sexual differences in photosynthesis and transpiration have been observed (Dzhaparidze 1969), analogous intraspecific adaptation to moisture regime could be anticipated. In *Rumex acetosella* neither photosynthetic nor conductance responses to moisture regime differed significantly between the sexes. Bourdeau (1958) also found no sexual differences in *Populus tremuloides* photosynthesis, but female aspens had significantly higher leaf respiration rates. Russian studies of carbohydrate metabolism (Sistev and Sizov 1971) and water relations (Sistev and Sizov 1972) under water stress indicate males of a variety of dioecious plants, including *R. acetosella,* are more drought resistant than females. The greater drought tolerance of male *Rumex acetosella* may be better explained by sexual differences in the respiratory metabolism underlying the growth process. If this is the case we can predict that under moisture stress male plants will have greater rates of growth respiration and possibly lower rates of maintenance respiration (Lechowicz et al. 1980).

The greater drought tolerance of male *Rumex acetosella* plants might also be explained, at least in part, by sexual differences in biomass allocation. Wallace and Rundel (1979), for example, reported distinctive male and female allocation patterns in the desert evergreen shrub, *Simmondsia chinensis,* that would lead to increased male tolerance of extreme drought stress. Across our experimental moisture gradient *R. acetosella* males allocated proportionately more biomass to living leaves than did females; males also have a related tendency to allocate more biomass to root tissue. Putwain and Harper's (1972) results for *R. acetosella* in North Wales are similar. Potter and Jones (1977) have shown that allocation to leaf tissue is more important than net assimilation rate in explaining the growth rates of herbaceous weeds. Since male and female plants of *Rumex acetosella* do not appear to differ in water use efficiency, the male with more leaf area per plant will have an advantage under water stress. On a per plant basis the male will fix more carbon and garner more of the limited water resource before it is locally exhausted; this disproportionate carbon gain under water stress provides an explanation for the observed male growth advantage under dryer conditions. These results emphasize the importance of comparing physiological traits in the context of adaptation coordinated at the whole plant level.

The patterns of biomass allocation in the sexes of *Rumex acetosella* under different moisture regimes cannot, however, be wholly typified by simple differences in root and leaf allocation. The multiple discriminant analysis shows that consistent differences in all biomass components contribute toward differentiating the sexes. Moreover this sex differentiation is maintained under all moisture regimes. This multivariate summary of allocation pattern offers a useful complement to the usual emphasis on reproductive effort (Gadgil and Solbrig 1971; Abrahamson and Gadgil 1973). In these experiments simple differences in reproductive effort could not be used to satisfactorily compare male and female *Rumex acetosella* because only some plants had begun to flower by the necessary harvest time. Nonetheless distinctive overall male and female allocation patterns were evident in the multiple discriminant analysis based on all biomass components.

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