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Abstract

Lindera melissifolia pondberry, is a federally endangered dioecious shrub found in Arkansas and four other southeastern states. Although by far the greatest area exists in Arkansas, it is broken into numerous small single-sex clones concentrated in two locations. Several stands have been lost during the 1980's according to records of the Arkansas Natural Heritage Commission. Even casual observation reveals that there are more males than females. This suggests dependence on vegetative reproduction, with possible bias against females. This paper reports on work investigating this suggestion. It was found that a 7:1 bias in area covered favors males. Poor survival of seedlings and transplants indicates that only apomictic reproduction is successful. Females allocate 45 times more resources to reproduction than males. Stem dieback occurs in both sexes but regrowth is vigorous. Shoot moisture stress and response of net photosynthesis and conductance favor growth of males.

Introduction

Dioecious plant species, in order to maintain sex ratios balanced near 1:1, must be able to compensate for the reproductive resource allocation devoted to production of fruits by females (Lloyd, 1974). In the shrubby New Zealand species *Hebe subalpina* females produce greater numbers of leaves early in the season (Delph, 1990). Leaves of female *Simmondsia chinensis* in the deserts of California and Baja California store more water, female plants allocate a greater proportion of growth resources to leaves, and females have a more open growth form. This results in females having enhanced potential for photosynthesis (Wallace and Rundel, 1979). In five wind-pollinated species of various growth forms in northern Utah, males predominate on xeric microsites and females on moister sites, thus minimizing competition between the sexes (Freeman et al., 1976). In *Lindera benzoin*, a close taxonomic and ecological relative of *Lindera melissifolia*, female growth and reproduction are favored in canopy gaps (Niesenbaum, 1992). In all the above dioecious species with the exception of *Lindera melissifolia*, sex ratios are 1:1.

Bias in sex ratios seems to be the exception among dioecious plants (Meagher, 1984). In one reported case, *Silene alba* populations in Massachusetts were female biased, especially in moister sites and at higher desities (Lovett Doust et al., 1987). *Acer negundo* populations showed a non-significant male bias, but there was no clear implication of reproductive resource allocation (Ramp and Stevenson, 1988).

Materials and Methods

Stand Characteristics.--In 1991 all the known stands of pondberry in Arkansas were surveyed. Each stand was found to be comprised of one or more single-sex groups of stems, referred to hereafter as clones. Limited excavation revealed stems to be connected by vigorous subsurface rhizones. This plus the single-sex grouping indicates that each clone is indeed a single biological individual. Occasionally isolated stems of one sex were found in a clone of the other sex, but usually clone boundaries were distinct. A number of clones were isolated from each other by distances of 10 to 100 m. Taken together, total area of all the known pondberry clones in Arkansas was 2.7 ha in 1991. Virtually all of this area was in two locations, one in the vicinity of Swifton in Lawrence County and the other northwest of Corning in Clay County. Although this survey revealed a number of previously unmapped clones, in 1992 additional clones were discovered in the vicinity of Swifton (Tom Foti, personal communication), but these additional clones are not covered in this paper. The clones known prior to 1992 are in 9 distinct stands: 3 in Clay County, 5 in Lawrence County, and a single clone in Woodruff County.

Based on census data taken during anthesis, area covered was biased toward males by a ratio of 7:1, with 86.7% male and 13.3% female. In several of the 9 stands there were only male plants. The stand with the greatest coverage of female plants had 42% females by area. Not only were female clones absent from some stands, they tended to be smaller than male clones in stands where both sexes were represented. There was a total of 42 female clones and 63 male clones, plus another 13 clones discovered after anthesis. Although no fruit was present

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on these 13, they could conceivably have been females too far from males for pollination to occur, so they were assigned to neither sex. Thus of the number of clones sexed, the ratio was 1.5: 1.0 in favor of males. Clones that had been sexed in previous years (Wright 1989a, b) had not changed in sex or location.

Stem density varied widely from clone to clone, ranging between 44 and 0.8 stems per m^2, but no sex difference in density was apparent. Typical densities in robust clones were 10-15 stems per m^2. Highest densities were in clones recovering from ground fire the year before. Densities below 5 stems per m^2 were associated with some form of disturbance, which could include water levels too high or too low, or heavy interference from shrubs and vines where canopy removal had occurred. Canopy closure ranged from 0-100%, with closure of 75% or more where no recent disturbance had occurred. Shrub-vine interference ranged from 0-85% of clone area, and was typically under 25% where no recent disturbance to the canopy had occurred. Of the 9 stands, 3 showed no disturbance to the forest, one had evidence of logging but not recently, one was undisturbed except for damage by ground fire, one showed some logging and windthrow, one had just been logged, one had been recently logged and drained, and one had excessively deep water during the growing season. In cases of recent logging, drainage, or deep flooding, stand condition was judged to be poor; thus four of the nine clusters were in poor condition.

Seedlings and transplants.--Regeneration of pondberry occurs naturally from vigorous rhizome sprouts (Wright, 1989b). Successful seed reproduction is rare, as indicated by the observation that plants seldom occur in mixed populations of both sexes, but seeds are viable and can be induced to germinate in nature by trampling them into a pond bottom when the pond is flooded (Wright, 1989b). Follow-up observations on the above experimental sowing revealed that the seed bank survived into the second season, when there was slight additional germination of trampled seed plus about 10% delayed germination of the untrampled control. Although seedling survival was excellent, seedlings grew very slowly, reaching an average height of 7.9 cm after two growing seasons. First year growth tended to die back, causing second year growth to originate near ground level and reach no higher than that of the first season. There was no indication when flowering would begin.

Transplantation of vigorous greenhouse-grown seedlings met with some success the first year (Wright, 1989b). By the end of the third growing season, however, only 3 of 61 transplants survived.

Predominance of vegetative reproduction was borne out by isozyme studies using starch gel electrophoresis. Comparisons among individuals of both sexes from the same stand and different stands revealed no isozyme differences for the 10 loci assayed. There were also several fixed heterozygotes, a condition not predicted when reproduction is sexual. These findings parallel those of Hooyschuur (1990).

Morphometric Comparisons.--Morphometric comparisons between sexes in adjacent pairs of clones revealed no discernable differences in leaf shape or stem height, but a tendency for males to have more leaves and branches per stem. In an earlier study, Richardson et al. (1990) found no significant morphometric differences between sexes except the production of fewer flowers and leaves by females. Richardson and Wright (unpubl. data) found morphometric differences between clones from different counties, but intersex difference was not studied.

Reproductive Resource Allocation.--Male bias in sex ratio can be taken as indicating effects of unequal reproductive resource allocation. Effects of unequal allocation would be amplified when reproduction was apomictic, since sexual regeneration would not be able to restore a 1:1 ratio.

Male stems produced more flowers than female stems, with mean dry biomass per stem 0.147 g for males and 0.067 g for females (n = 20). Mature fruit biomass, however, exceeded male flower biomass by over an order of magnitude, averaging 6.57 g per stem (n = 20). This means that total reproductive resource allocation was 6.64 g for females and 0.147 g for males, a ratio of 45:1. Although stems that had fruited often died back partially or completely the next growing season, dieback of male stems was also common. Measurements of dieback revealed nearly identical values for the sexes in proportion of stems dying back, length of dieback, and proportion of stems dead to the ground line.

Net Photosynthesis and Stomatal Conductance.--Gas exchange of carbon dioxide and water vapor was determined for 50 leaves of each sex in adjacent clones on five dates during the 1991 growing season, using a LICOR 6200 Portable Photosynthesis System. Data were analyzed with the SAS statistical package. The general linear models procedure revealed no significant differences between sexes in photosynthesis, light, temperature, or CO2, but a highly significant difference between sexes in stomatal conductance (Pr>F0.0007). Males had the higher conductance by about 20%. Multivariate ANOVA revealed a highly significant overall sex effect (Pr>F0.0001). Stepwise discriminate analysis gave rankings as follows:

	Partial R2	Prob. > F
1 Conductance	.0231	.0007
2 Temperature	.0189	.0021
3 Photosynthesis	.0082	.0431

Higher stomatal conductance for males indicates that transpiration occurs at a greater rate, and implies that net photosynthesis could also be greater since both processes depend upon adequate numbers of stomates and stomatal aperture. Under field conditions, however, net photosynthesis did not differ between sexes.

Shoot Water Potential.--Under typical field conditions, soil moisture remains adequate for most of the growing season in the seasonal ponds where pondberries are found. This suggests that shoot water potential (WP) remains high, and such was the case in 1991. At the site and dates of gas exchange determination, mean shoot water potential was measured on samples of 5 to 10 detached shoots of each sex using a PMS pressure bomb. Mean WP never fell lower than - 1.27 MPa. Although sample sizes were too small to yield significant differences, male WP was lower than female WP on each date, with differences ranging from 5 to 30%.

Discussion

Unequal reproductive resource allocation in a female: male ratio of 45:1 suggests that to maintain a 1:1 sex ratio, *Lindera melissifolia* would need to possess compensating properties in its species biology favoring females. This study has revealed little evidence of such compensating properties. The smaller number and size of female clones indicates that reproductive load on females is not compensated. Absence of females from some stands implies their elimination from these stands. Where both sexes were present, this study found no differences in stem density; however, Richardson et al. (1990) did find a distinction, with female stem density only 60% that of male stem density. Taken as a whole, the above evidence indicates that females have suffered a decline disproportionate to that of males in this endangered species.

There is only limited evidence for genetic distinctions between the sexes. Morphometrically, females have lower numbers of leaves and flowers. Limited electrophoretic data revealed no differences between sexes, and indeed no differences at all among the populations of several stands. This plus the presence of fixed heterozygotes is consistent with the thesis that apomictic reproduction predominates. There is also no evidence that females can photosynthetically compensate for unequal reproductive resource allocation. Net photosynthesis was the same between the sexes, while stomatal conductance was higher in males. If anything, this bias in conductance should favor male photosynthesis, although a compensating bias may occur during infrequent late-season drought stress as recorded by Wright (1989a). Males would be expected to suffer drought stress sooner due to their higher stomatal conductance.

If sexual reproduction were competent, this would be expected to reduce the bias in sex ratio, but the species does not produce significant numbers of seedlings under natural conditions. Growth of the few seedlings is slow, unable to compete effectively with apodictic regeneration from rhizome sprouts. Shoots die back readily, and although new rhizome sprouts appear abundant, females clearly have not maintained themselves by this means. In contrast, the ubiquitous *Lindera benzoin*, with a 1:1 sex ratio (Niesenbaum, 1992), does not appear to spread by rhizome sprouts into large single sex clones. Although *Lindera benzoin* females can produce numerous fruits, they do it on much larger and longer-lived stems that apparently are able to survive the burden of unequal reproductive resource allocation.

Failure of females to maintain themselves in natural populations is likely accelerating the demise of this endangered species, *Lindera melissifolia*. As Wright (1989a) has previously suggested, only managed sexual reproduction has the potential of maintaining females and thus the biological integrity of the species.

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