

SEXUAL DIMORPHISM AND COST OF REPRODUCTION IN THE DIOECIOUS SHRUB *LINDERA BENZOIN* (LAURACEAE)¹

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We examined sexual dimorphism in reproductive allocation and its effects on growth and subsequent reproduction in a natural population of the dioecious woody shrub *Lindera benzoin* (L.) Blume. In addition to comparing natural patterns of growth and reproduction in a marked population of 251 females and 87 males, we experimentally examined the effect of reduced reproduction on future growth and reproduction in female plants and examined sexual dimorphism in carbon/nutrient balance. Our results suggest that females of *L. benzoin* bear greater reproductive costs in terms of both biomass and minerals. These costs were measurable in terms of current biomass and allocation of nitrogen to reproduction, as well as subsequent growth, reproduction, and tissue carbon/nutrient balance. Based upon the results of a fruit-thinning experiment and path analysis, fruit production in 1991 appeared to have direct negative effects on fruit production in 1992, an effect that was not necessarily mediated through effects on plant growth. We discuss our results in the context of other intrinsic and extrinsic factors that can influence growth, reproductive costs, and mortality in this species.

Life-history theory in plants depends heavily upon assumptions of trade-offs between reproductive and vegetative processes (cf. Lovett Doust and Lovett Doust, 1988). Clear demonstration of such trade-offs has been problematic, despite strong correlative evidence derived from years of field study. Research on dioecious species has largely focused on the documentation of differences between the sexes in life-history and resource allocation patterns. Differences between the sexes have often been attributed to reproductive dimorphisms that influence the relative allocation of resources to reproduction, growth, and maintenance (Lloyd and Webb, 1977; Bierzychudek and Eckhart, 1988). A common specification of this concept is that, because of fruit production, females should allocate a greater proportion of assimilated resources to reproduction. As such, females are expected to suffer greater costs in terms of growth and future reproduction under conditions of resource limitation.

Despite some exceptions (cf. Mitton and Grant, 1980; Sakai and Burris, 1985; Pendleton et al., 1992), a majority of studies have documented relatively greater reproductive effort in terms of biomass, energy, and/or minerals for females (Bullock and Bawa, 1981; Gross and Soule, 1981; Meagher, 1984; Cavigelli et al., 1986; Lovett Doust, O'Brien, and Lovett Doust, 1987; Agren, 1988; Allen and Antos, 1988; Antos and Allen, 1990; Cipollini and Stiles, 1991; Korpelainen, 1992). Yet, relatively few studies of dimorphic species have attempted to quantify the effects

of current differential reproductive allocation on future growth and reproduction (but see Putwain and Harper, 1972; Meagher and Antonovics, 1982; Agren 1988; Bullock, 1992; Vasiliauskas and Aarssen, 1992). And with a few exceptions (e.g., Delph, 1990; Ashman, 1992), studies that have attempted to experimentally assess costs of reproduction have utilized artificial increases in fruit set (seed/ovule ratio) of hermaphroditic species (usually via hand-pollination) as a means of temporarily increasing reproductive costs (for review of hand-pollination studies, see Young and Young, 1992). In addition to technical problems of implementation (cf. Zimmerman, 1988), the use of pollen augmentation would seem to be of limited value for plants with primarily resource-limited reproduction, as they should not be expected to respond readily to hand-pollination with increased fruit set (Bloom, Chapin, and Mooney, 1985). Such studies have been largely restricted to herbaceous woodland perennials that are commonly pollen-limited, at least over the short-term (e.g., Primack and Hall, 1990). Others have examined the relationship of natural variation in fruit set on vegetative growth (e.g., Reekie and Bazzaz, 1987), or the effects of reproduction on subsequent fates of sexually labile plants (e.g., Bierzychudek, 1984; Schlessman, 1991). While some of these studies have reported evidence supporting the concept of cost of reproduction (e.g., Bierzychudek, 1984; Snow and Whigham, 1989; Zimmerman and Aide, 1989; Ackerman and Montalvo, 1990; Primack and Hall, 1990), others have reported equivocal results (e.g., Reekie and Bazzaz, 1987; Horvitz and Schemske, 1988a, b). Longitudinal studies of reproductive and vegetative dimorphism in dioecious species, coupled with experimental alterations in flowering and fruit set, provide alternative avenues for assessing potential tradeoffs between growth and reproduction (Ashman, 1992). Dioecy is particularly common in fleshy-fruited, woody perennials (Bawa, 1980; Givnish, 1980; Meuncho, 1987). Such analyses should account for plant size-dependent effects, because such effects can be a major source of variation in growth and reproduction in flowering plants (Herrera, 1991) and thus

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have the potential to influence relative costs of reproduction (e.g., Lawrence, 1993).

Differential costs of reproduction may have other, more subtle consequences for dioecious plants. According to the carbon/nutrient balance hypothesis (Coley, Bryant, and Chapin, 1985), nitrogen limitation should lead to increased allocation to C-based secondary compounds in plants. The assumption that females of dioecious plants bear greater reproductive costs in terms of nitrogen leads to the expectation of lower tissue nitrogen levels, higher levels of C-based secondary compounds, and concomitantly increased levels of antiherbivore defense in females (Elmqvist et al., 1988; Jing and Coley, 1990; Niesenbaum, 1992b). Thus, in addition to its potential influence on patterns of growth, sexual dimorphism in costs of reproduction has potential effects on levels of nitrogen and C-based secondary compounds of tissues, which in turn has potential implications for population demography through effects on plant quality for herbivores.

We sought to examine sexual dimorphism in reproductive allocation and its effects on growth, carbon/nutrient balance, and subsequent reproduction in the dioecious woody shrub *Lindera benzoin* (L.) Blume. To accomplish this, we addressed the following specific questions: 1) How do estimates of biomass and nutrient allocation to reproduction differ between females and males?, 2) Do patterns of growth in ramets, leaves, and newly produced shoots differ between females and males?, 3) Do patterns of carbon/nutrient balance, as estimated from nitrogen and total phenolic content, differ in leaf and shoot tissues of females and males?, and 4) Does a reduction in reproductive effort by females in one year lead to enhanced reproduction and/or growth in later time periods?

MATERIALS AND METHODS

Study species—Individuals (genets) of the perennial woody shrub *L. benzoin* bear functionally unisexual flowers (Boyle, 1980) and reproduce vegetatively via the initiation of clusters of stems (ramets) from a central root bole. At the site of our study, Smithsonian Environmental Research Center (SERC), Edgewater, Maryland, the shrubs are concentrated in moist locations within understories of mid- to late-successional riparian and floodplain "tulip poplar association" forests (Brush, Lenk, and Smith, 1980), whose overstories are typically dominated by *Liriodendron tulipifera* L., *Quercus* spp., *Carya* spp., and *Liquidambar styraciflua* L. The site of our primary study population, Fox Point, SERC (hereafter Fox Point) is a midsuccessional site (about 40 yr since abandonment as a cattle pasture, D. Higman, SERC, personal communication) occurring on Aldelphia sandy loam (2%–5% slope; Kirby and Matthews, 1973). During the years of this study (1991 to 1993), this site was dominated by *L. tulipifera* L., *L. styraciflua* L., and *Prunus serotina* L. in the overstory and was occupied almost exclusively by *L. benzoin* in the shrub layer.

Shade tolerance has been considered an important factor influencing distribution and growth in *L. benzoin*, although individual shrubs show substantial growth responses to light level variation (Moore and Willson, 1982; Veres and Pickett, 1982; Niesenbaum, 1992b; M. Cipolini, D. Wallace-Senft, and D. Whigham, unpublished

data). At SERC, plants flower when leafless in early April, followed by leaf and shoot initiation in early May. Female flowers consist of clusters of two to five unilocular ovules borne on short peduncles, and male flowers consist of morphologically similar clusters of two to five florets containing ten to 20 anthers in each floret. Occasional bisexual and sexually inconstant plants have been noted in *L. benzoin* (cf. Primack, 1985). The frequency of putative bisexual plants was low in and around the Fox Point population (<1%), and sex expression of individuals was constant for the period of this study. For purposes of this study, we classified morphologically bisexual individuals as females. Both female and bisexual plants produce single-seeded fleshy fruits that are dispersed primarily by birds (Stiles, 1980; Johnson et al., 1985).

Census methods—In order to compare growth and reproductive patterns of adult female and male *L. benzoin*, we initiated an annual census at Fox Point in 1991. In early April of that year, we randomly selected 30 female and 20 male genets, and tagged all living ramets within each genet. Genets were classified as adults if they had produced at least one flowering ramet. Flower numbers were estimated by multiplying the number of flower clusters on each ramet by the mean number of ovules (females) or florets (males) per flower cluster, as determined from counts of 20 clusters per plant. Bulk samples of about 500 flower clusters were collected from plants in the immediate vicinity of the censused plants, oven-dried at 60 C, and weighed for determination of dry mass (g) per ovule (females) or floret (males) and for nitrogen analysis. In June 1991, all presumed adult genets (largest ramet > 1 m in height) within a 30 × 50-m area were mapped by triangulation from known points within a 5 × 10-m grid system superimposed on the population. Individual genets were tagged, and the basal diameters (at a height of 5 cm) of all ramets were measured using vernier calipers. In August 1991, 96 large juveniles (largest ramet 0.5 to 1 m in height) were similarly mapped and measured. Subsequent growth analyses revealed that nearly all height and basal diameter growth of mature ramets occurs prior to the end of June. We thus considered August 1991 data equivalent to those collected in June 1991.

In late August 1991, mature fruits were censused on all plants. Initial flower number for each female that lacked 1991 floral census data was estimated using the mean fruit set value (0.140 ± 0.018 mature fruits per ovule) for the 30 females with full flower and fruit censuses. Initial flower number for each male that lacked 1991 floral census data was estimated using the regression of flower number (FLS) on genet volume (GNVOL): $FLS = (GNVOL \times 3.950) + 54.15$ ($R^2 = 0.753$, $df = 18$, $P \leq 0.05$).

As a means of comparing modular growth parameters of females and males, we collected and measured newly produced shoots. In September 1991, newly produced terminal (< 1 yr old) shoots and leaves were sampled from 20 randomly selected genets of each sex. For each shoot, we counted all leaves and determined leaf area (cm²) using a LI-COR Model 3100 area meter. We measured shoot basal diameter (mm) and length (cm), and determined leaf and shoot mass (g) before and after oven-drying at 60 C. For each shoot sample we calculated dry mass (g) per leaf and area (cm²) per leaf.

In April 1992, we censused flowers on all males and on the 30 females with full flower census data for 1991. All plants initially marked as large juveniles in August 1991 flowered at this time ($N = 66$ females and 30 males). As of this census, there were 251 female and 87 male genets in the population. For all female genets, we counted ovules on two randomly selected branches containing approximately 50 to 200 ovules and marked these branches for later use in estimating fruit set (seed/ovule ratio).

In June 1992, we remeasured basal diameter and height of each living ramet. Using these data, we estimated the following size and growth parameters for the largest ramet within each genet for the June 1991 to June 1992 period: height increase (cm), basal diameter increase (cm), ramet volume (cm^3), and ramet volume increase (cm^3). Ramet volume (RV) was estimated using the equation for a cylinder: $RV = (\text{PI} \times R^2 \times H)/3$; where $\text{PI} = 3.1416$, R = ramet basal radius, and H = ramet height. For each genet, we also estimated genet volume (GNVOL; cm^3) and genet volume increase (cm^3), by summing the volumes of the individual ramets. Values for growth parameters were calculated as the difference between the log-transformed final and initial measurements.

In August 1992, we censused fruits on all females. We then determined mean fruit set by dividing the number of mature fruits on marked branches by the initial ovule number on those branches. Initial ovule number (FLS) for each ramet was estimated from the mature fruit number (FRTS) and the mean seed/ovule ratio (FS): $\text{FLS} = \text{FRTS}/\text{FS}$. Flower number could not be estimated in this manner for ten small plants with no fruit set; for these plants, initial flower number was estimated using the linear regression of ovule number on genet volume: $\text{FLS} = (\text{GNVOL} \times 0.768) + 0.001$ ($R^2 = 0.80$, $df = 250$, $P < 0.0001$).

In September 1992, we again sampled shoots and leaves for morphometric and biomass determination and for chemical analysis. From the largest ramet within 36 male and 36 female genets, we measured newly produced terminal shoots, and collected ten newly produced lateral shoots. Position within plants for lateral shoots was standardized by restricting collection to shoots on side branches of the main axial ramet. Measurements followed those made in 1991, except that leaf area was not determined. Leaf and shoot samples were ground to pass a #40 mesh using a Wiley Mill and were stored at -20°C in capped polyethylene vials prior to chemical analysis.

Fruit-thinning experiment—As a means of assessing costs of reproduction in terms of future growth and reproduction, we conducted an experiment using 30 medium-sized females (basal diameter of largest ramet 2–4 cm) in an area adjacent to the mapped population. In early June 1991, we measured basal diameter and height of each ramet, and censused immature fruits. Individual fruit biomass was about one-fifth that of mature fruits at this time (mean = 0.0199 g for aborted fruits vs. 0.1228 g for mature fruits), and mineral nutrient concentrations were nearly identical to those of mature fruits (M. Cipollini, unpublished data). For 15 randomly selected individuals, we thinned 50% of immature fruits by hand-removal of every other fruit along all reproductive branches. Subsequent size, fruit, and flower censuses were

conducted as for the main population. We estimated subsequent fruit abortion by dividing the difference between the initial and final fruit number by the initial fruit number.

Fruit-thinning has the potential to reduce subsequent rates of abortion and to increase allocation per individual fruit (Stephenson, 1981). To address this issue, in September 1991, we sampled 50 mature fruits from each of the 30 shrubs and from a random subset of 30 shrubs in the main study population. For biomass determinations, fruits were first weighed whole; then the fruit pulp was removed from each seed and the fresh seed mass was determined. Wet pulp mass was calculated as the difference between wet whole fruit mass and wet seed mass. Seeds and pulp samples were oven-dried to constant mass at 60°C and re-weighed. Dry pulp mass was estimated from the calculated wet pulp mass and its moisture content, a method that accounts for pulp lost during separation. Pulp and seed samples were ground and stored in a manner identical to leaf and shoot samples.

Chemical analyses—For each pulp and seed sample collected in 1991 and for each leaf and shoot sample collected in 1992, we determined total nitrogen (% dry mass) using micro-Kjeldahl digestion followed by autoanalysis (Williams, 1984). For each pulp and seed sample, we also determined P, K, Ca, Mg, Mn, B, Cu, Fe, Al, Zn, and Na using inductively coupled plasma emission spectroscopy. We estimated total phenolics for leaf and shoot samples collected in 1992 using 95% methanol extraction for 1.5 hr at 23°C , followed by the Prussian Blue colorimetric assay (0.016 M potassium ferricyanide + 0.1 M ferric ammonium sulphate; Budini, Tonelli, and Giorotti, 1980; Graham, 1992). Results were expressed as mg/g dry mass catechin equivalents. The Prussian Blue assay, like the analogous Folin-Denis assay for total phenolics (Harborne, 1989), is subject to error if phenolic profiles differ strongly among samples (i.e., if there are differences among samples in the relative concentrations of individual compounds). Thus, we qualitatively characterized the phenolic profiles for bulk samples of male and female tissues using thin-layer chromatography (silica gel medium; toluene-acetone-formic acid solvent [6:6:1]; Prussian Blue developer; A. Hagerman, Miami University, Miami, OH, personal communication). Because thin layer chromatograms showed no differences in phenolic profiles of plants from the two sexes, we assumed that the results of the total phenolic assay were representative of relative quantitative differences.

Statistical analyses—Univariate and multivariate analyses-of-variance and-covariance (ANOVA, ANCOVA, MANOVA, MANCOVA; PROC GLM, SAS Institute, Inc., 1982) formed the primary statistical analyses designed to quantify effects of sexual dimorphism (main effect = sex) and fruit-thinning (main effect = treatment). The ANCOVA approach is analogous to ANOVA using relative growth data, in that it helps to account for size-dependent effects, but ANCOVA avoids problems associated with the statistical analysis of ratios. Covariates included initial size estimates for growth variables (e.g., ramet height in the analysis of ramet height increase), and covariates for MANCOVA analyses employed the plant

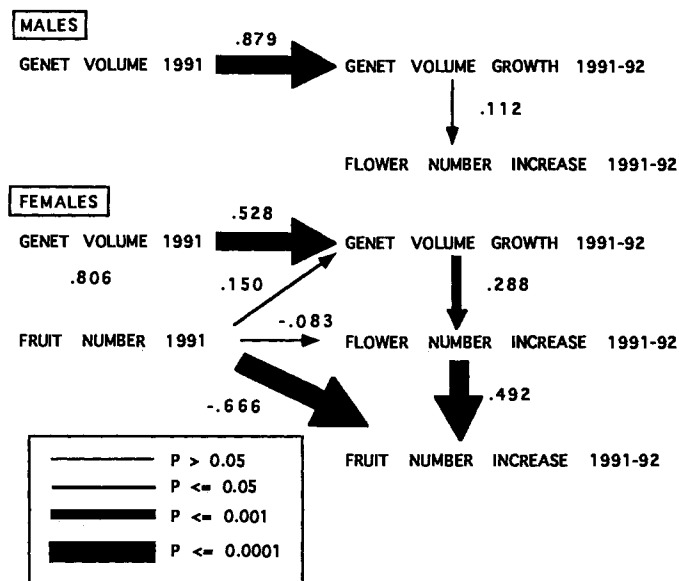


Fig. 1. Path analysis diagram describing flowering, growth, and fruit production in male and female *Lindera benzoin*, Fox Point, SERC, 1991–1992. Numerical values for each of the paths are standardized regression coefficients.

size variable best correlated with the dependent variables (e.g., genet volume in the analysis of reproductive variables). In order to normalize distributions, size data were log-transformed and percentage data were arcsine-transformed prior to analysis. For analyses involving individual ramets (e.g., ramet basal diameter increase), we present results of analyses for only the largest ramet within each genet. Because of unbalanced designs and the need to make tests invariant to the ordering of the main effects, all *F*-tests were based upon type III (partial) sums of squares (PROC GLM, SAS Institute, Inc., 1982). Upon finding significant overall effects in multivariate tests, we used separate ANOVA and ANCOVA analyses to identify individual variables that differed significantly between sexes or treatments.

Additionally, we conducted a path analysis (cf. Schemske and Horvitz, 1988) designed to assess the direct and indirect effects of plant size and fruit production on growth,

flowering, and fruit set. This analysis was based upon standardized regression coefficients corresponding to the linear equations of the putative causal paths illustrated in Fig. 1 (PROC REG: SAS Institute, Inc., 1982), using data for the 30 females and 20 males with full flower censuses. For more complete discussions of path analysis, including its advantages, limitations, and extensions, see Li (1981), Mitchell (1992), and Hayduk (1987).

Whole ramet and genet growth and reproduction.—Based upon tabulation of all reproductive plants, adult genet sex ratio was highly female-biased at Fox Point ($N = 251$ females, 87 males, sex ratio = 0.34). According to ANOVA analyses, ramet basal diameter, height, volume, genet volume, total number of ramets, and number of new ramets did not differ significantly (main effect = sex; all *F* values < 3.10, *df* = 1/336, all *P* values > 0.08) between the sexes in 1991 or in 1992 (Table 1).

Estimates of total reproductive biomass and reproductive nitrogen were much higher for females in both years (Table 1). These estimates were based upon extrapolation from biomass and nitrogen determinations on subsamples, and in part from flower number estimates in 1991; therefore, no statistical analyses were applied. Additionally, we made the simplifying assumption that aborted ovules (lacking pollination) and aborted fruits (pollinated, but dropped prior to maturation) were identical in biomass and nitrogen content. As such, our estimates for reproductive biomass and nitrogen may be slightly elevated for females. Nevertheless, the differences between females and males are large enough to make clear the degree of within-year differential reproductive allocation in *L. benzoin*. Assuming that females and males allocate biomass and minerals equally to vegetative growth (see results below), we arrive at estimates of about 14–18 times greater biomass allocation and 11–12 times greater nitrogen allocation to reproduction for females.

Growth in ramet diameter, height, volume, and genet volume was slightly higher for males (Table 2). Separate ANCOVA analyses indicated that differences between the sexes were significant only for ramet volume and genet volume increase. Net ramet number increase (1991 to 1992) was slightly higher for females, but based upon ANCOVA (covariate = genet volume 1992), the difference was not significant (Table 2). Although terminal stem

TABLE 1. Plant size and reproductive data for *Lindera benzoin*, Fox Point, SERC, 1991–1992.^a

Variable	Year = 1991		Year = 1992	
	Females ^b	Males ^b	Females ^b	Males ^b
Ramet basal diam (cm)	1.89 (0.05)	1.83 (0.10)	2.07 (0.05)	2.03 (0.11)
Ramet height (cm)	233.63 (5.67)	230.18 (11.02)	269.45 (5.90)	270.11 (11.54)
Ramet volume (cm ³)	336.52 (31.71)	377.81 (77.38)	443.49 (37.23)	500.06 (94.59)
Genet volume (cm ³)	443.82 (43.11)	569.72 (140.5)	587.55 (52.27)	755.65 (170.4)
Total ramets	3.28 (0.14)	3.56 (0.32)	4.46 (0.20)	4.36 (0.39)
New ramets	0.48 (0.08)	0.64 (0.14)	1.49 (0.17)	0.83 (0.16)
Flowers	1,593.2 (151)	2,234.7 (561)	2,126.8 (288)	2,834.3 (575)
Aborted ovules/fruits	1,436.8 (131)	—	1,897.9 (266)	—
Mature fruits	156.37 (25.62)	—	228.89 (30.88)	—
Reproductive biomass (g)	47.59 (5.37)	3.37 (0.85)	68.20 (8.66)	3.77 (0.95)
Reproductive nitrogen (g)	1.76 (0.18)	0.15 (0.04)	2.50 (0.31)	0.22 (0.04)

^a Diameter, height, and volume are for the largest ramet within each genet; other data refer to the whole genet ($N = 251$ females, 87 males). Estimates for reproductive parameters represent extrapolations from censuses on 30 females and 20 males in 1991, and from censuses of all adults in 1992.

^b Means, with standard errors in parentheses.

TABLE 2. Plant size and reproductive increases for *Lindera benzoin*, Fox Point, SERC, 1991–1992.^a

Parameter	Females ^c	Males ^c	Main effect ^b	
			Sex	Covariate
Basal diam increase (cm)	0.18 (0.01)	0.20 (0.01)	0.88	16.60****
Height increase (cm)	35.82 (1.33)	39.93 (1.81)	2.29	89.70****
Volume increase (cm ³)	106.98 (8.63)	122.25 (19.24)	4.99*	67.05****
Genet volume increase (cm ³)	143.73 (12.12)	185.93 (33.37)	8.44**	70.54****
Net ramet number increase	1.18 (0.16)	0.79 (0.18)	0.52	4.62*
Flower number increase	533.66 (244.61)	599.59 (274.83)	10.66**	55.95****

^a Diameter, height, and volume are for the largest ramet within each genet; other data refer to the whole genet ($N = 251$ females and 87 males for growth parameters, $N = 30$ females and 20 males for flower production).

^b F -values resulting from ANCOVA analyses (covariates = initial values for each growth parameter; $df = 1/335$ for growth parameters and 1/48 for flower number).

^c Means, with standard errors in parentheses.

* $P \leq 0.05$; ** $P \leq 0.01$; **** $P \leq 0.0001$.

sampling has the potential to affect subsequent resource allocation patterns and growth, the results of these and related statistical analyses were not substantively affected by the exclusion of the 40 individuals that were so sampled in 1991. In all sampled plants, the subterminal (lateral) branch took over as the main terminal stem in the year following stem sampling.

Regression analyses revealed that ramet volume increase and genet volume increase was linearly related to initial ramet volume and genet volume, respectively (Table 3), which is indicative of size-dependent growth. Increases in ramet diameter, height, and number were unrelated to initial ramet diameter, height, and number, respectively (results not shown). Based upon homogeneity-of-slopes tests, the slope of the relationship of initial ramet volume to ramet volume increase was significantly higher for males (Table 3).

Although flower numbers (Tables 1, 4) and flower number increases (Table 2) were slightly higher for males, the relationship of flower number to genet volume was identical for males and females in both years (Table 3). ANCOVA analyses (covariate = genet volume) indicate that flower number was only marginally significantly different between males and females in 1992 (main effect = sex: $F = 3.56$, $df = 1/334$, $P = 0.0600$), suggesting that differences in rates of flower production are primarily a consequence of differential growth.

The standardized regression coefficients resulting from path analysis (Fig. 1) indicate strong direct effects of plant size (genet volume) on subsequent growth (genet volume increase) for both males ($R^2 = 0.879$) and females ($R^2 = 0.528$). A strong direct negative effect ($R^2 = -0.666$) was found for the path leading from fruit number in 1991 to fruit number increase in 1992. The indirect path from

genet volume in 1991 to fruit number increase in 1992 (which passes through genet volume increase and flower number increase) is the most important overall path (sum of the path coefficients = 1.308). The direct effect of fruit number in 1991 on fruit number increase ranks as the second most important individual parameter for females. In short, upon accounting for indirect effects of changes in plant size on flower and fruit production, the results of the path analysis suggest strong direct negative effects of fruit production in 1991 on fruit production in 1992.

Modular leaf and shoot growth—Examination of individual characteristics reveals that parameters describing shoot and leaf growth were slightly higher for males in both years. These differences are illustrated by results for terminal shoots in 1991 (Table 4). Results for terminal stems in 1991 showed similar, although nonsignificant differences between the sexes, while differences between the sexes were significant ($P \leq 0.05$) for all measured parameters for lateral shoots in 1992 (data not shown). MANOVA analyses indicated significant overall differences between the sexes in terminal leaf and shoot growth in 1991 and lateral leaf and shoot growth in 1992 (Table 5). Differences were not significant for terminal shoots in 1992, although the MANOVA for terminal shoot characters in 1992 was marginally significant ($P = 0.0576$).

In these comparisons, flower number per shoot was consistently slightly higher for males. Based upon homogeneity-of-slopes tests, the regression of flower number on lateral shoot mass was significant (effect of shoot mass: $F = 606.38$, $df = 1/719$, $P < 0.0001$), and similar for both sexes in 1992 (main effect = sex: $F = 2.79$, $df = 1/719$, $P = 0.0950$; sex \times shoot mass interaction effect: $F = 0.03$, $df = 1/719$, $P = 0.8558$). Similar results were

TABLE 3. Linear regressions describing growth and reproduction of *Lindera benzoin*, Fox Point, SERC, 1991–1992.^a

Independent variable	Dependent variable	Females			Males		
		Slope	Inter.	R^2	Slope	Inter.	R^2
Ramet volume increase	Ramet volume (cm ³) 1991	0.148	55.92	0.31	0.216	41.21	0.75*
Genet volume increase	Genet volume (cm ³) 1991	0.197	56.40	0.49	0.207	65.89	0.76
Flower number 1991	Genet volume (cm ³) 1991	2.165	-6.30	0.61	2.171	-6.51	0.74
Flower number 1992	Genet volume (cm ³) 1992	1.529	-3.00	0.62	1.378	-1.55	0.67

^a Regressions for flower number in 1991 are based on the 30 females and 20 males for which full census data were available; other analyses use the entire sample ($N = 251$ females and 87 males). Variables were log-transformed prior to analysis. Asterisks denote significant differences ($P \leq 0.05$) between slopes for females and males, based upon homogeneity-of-slopes tests.

TABLE 4. Terminal shoot characteristics of male and female *Lindera benzoin*, Fox Point, SERC, October 1991.^a

Parameter	Females	Males
Length (cm)	39.90 (1.89)	51.93 (2.85)***
Basal diam (cm)	0.35 (0.02)	0.51 (0.34)***
Shoot mass (g)	1.19 (0.13)	2.82 (0.39)***
Shoot volume (cm ³)	2.69 (0.21)	6.39 (0.73)***
Leaf number	12.45 (0.73)	15.00 (0.78)*
Total leaf mass (g)	1.93 (0.17)	3.24 (0.30)***
Mass (g) per leaf	0.16 (0.01)	0.21 (0.01)**
Total leaf area (cm ²)	727.21 (60.30)	1,168.24 (102.8)**
Area (cm ²) per leaf	60.63 (5.67)	75.58 (3.46)*
Flower buds per shoot	14.50 (1.51)	22.85 (3.57)*

^a Means (with standard errors in parentheses) for samples taken from 20 individuals. Asterisks denote significant effects of sex in univariate tests (ANOVA, or ANCOVA in the case of flower buds per shoot; covariate = shoot mass).

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

obtained in analyses of terminal shoots in 1991 and 1992 (data not reported); thus, slight differences in the numbers of flowers produced by males and females were primarily a function of differences in shoot growth. These results support those obtained from analyses of whole genet growth and flower production.

To account for potential tradeoffs between shoot growth and new shoot initiation, we also compared the number of lateral branches on 1-yr-old stem segments subtending the terminal shoot of each plant. ANCOVA analysis (covariate = basal diameter 1992) indicated that the sexes did not differ in rates of lateral branch production (main effect = sex: $F = 0.85$, $df = 1/69$, $P = 0.3610$).

We found a significant effect of plant sex on leaf chemistry in 1992, which was primarily the result of significantly higher leaf phenolics in females (Table 6). Shoot nitrogen was only marginally significantly lower in females ($P = 0.0686$), while the sexes had identical concentrations of shoot phenolics. MANOVA analyses indicated overall significant differences between the sexes in leaf growth and chemistry (Table 5) with a marginally significant trend ($P = 0.0645$) for differences in shoot chemistry.

Fruit thinning experiment—The 50% fruit thinning treatment produced slight increases in biomass (Table 7)

TABLE 5. MANOVA results: terminal and lateral shoot characters of male and female *Lindera benzoin*, Fox Point, SERC, 1991 and 1992.^a

Variables	Wilks' Lambda	df	F
Terminal shoots (1991 and 1992): ^b			
Leaf characters 1991	0.5764	5/34	3.72**
Stem characters 1991	0.6431	4/35	3.05*
Leaf characters 1992	0.9891	3/68	0.23
Stem characters 1992	0.8707	4/67	2.45
Lateral shoots (1992): ^c			
Leaf number, mass and area	0.8553	3/68	3.85*
Shoot morphology and mass	0.8368	5/66	2.56*
Leaf number, mass and chemistry	0.7307	5/34	2.51*
Shoot mass and chemistry	0.7814	4/35	2.45

^a Main effect = plant sex.

^b For variable identities and means, refer to Table 4.

^c For variable identities and means, refer to Tables 4, 6.

* $P \leq 0.05$; ** $P \leq 0.01$.

TABLE 6. Chemical characteristics of leaves and shoots of male and female *Lindera benzoin*, Fox Point, SERC, September 1992.^a

Parameter	Females	Males
Leaf nitrogen ^b	3.22 (0.07)	3.31 (0.06)
Shoot nitrogen ^b	0.92 (0.03)	0.97 (0.03)
Leaf phenolics ^c	1.23 (0.04)	1.06 (0.04)*
Shoot phenolics ^c	0.76 (0.04)	0.76 (0.03)

^a Means, with standard errors in parentheses, for shoots sampled from 36 individuals of each sex. Asterisks denote significant effects of sex in one-way ANOVA.

^b Percent dry mass total Kjeldahl nitrogen.

^c mg/g dry mass catechin equivalents.

* $P \leq 0.05$; ** $P \leq 0.01$.

and slight increases in the content of most minerals in pulp and seeds (Table 8). Despite these trends, the results of MANOVA analyses indicated no significant effects of fruit thinning on pulp and seed biomass (main effect = treatment: Wilks' Lambda = 0.8176, $F = 1.971$, $df = 6/53$, $P = 0.0864$) or on pulp and seed mineral content (Wilks' Lambda = 0.1333, $F = 1.354$, $df = 24/5$, $P = 0.3980$ for percent dry mass; Wilks' Lambda = 0.1053, $F = 1.769$, $df = 24/5$, $P = 0.2743$ for absolute content per fruit). Patterns of mineral allocation did differ strongly between pulp and seed tissues. Most notably, there was much higher K and Na in pulp tissues and higher levels of most other minerals, especially N, in seed tissues (Table 8).

Fruit abortion in the period following fruit thinning in 1991 was slightly, but nonsignificantly, lower for treatment plants (Table 9). Subsequent levels of growth, flowering, and fruit set also appeared to be somewhat higher for hand-thinned plants, but ANCOVA analyses indicated that only total fruit production in 1992 differed significantly from that of control plants (Table 9). Results of MANCOVA for ovule and fruit production in 1992 were significant for both the fruit thinning treatment effect (Wilks' Lambda = 0.8255, $F = 3.49$, $df = 2/33$, $P < 0.05$) as well as the plant size covariate (Wilks' Lambda = 0.7155, $F = 6.56$, $df = 2/33$, $P < 0.05$). These results, coupled with the observation of significant covariate effects in the individual ANCOVA analyses (Table 9), indicated that differences in growth and flower production between hand-thinned and control plants could, in part, be explained by variation in plant size (genet volume). However, upon controlling for plant size effects, fruit production in 1992 was still significantly higher for plants that were hand-thinned in 1991 (Table 9).

TABLE 7. Effect of fruit thinning on pulp and seed mass (g) of *Lindera benzoin*, Fox Point, SERC, 1991.^a

Parameter	Hand-thinned	Control
Wet mass per fruit pulp	0.1815 (0.0062)	0.1857 (0.0041)
Dry mass per fruit pulp	0.0468 (0.0022)	0.0441 (0.0012)
Wet mass per seed	0.1053 (0.0030)	0.1041 (0.0026)
Dry mass per seed	0.0786 (0.0022)	0.0769 (0.0019)
Dry mass per whole fruit	0.1254 (0.0035)	0.1211 (0.0028)

^a Means, with standard errors in parentheses, for samples of 50 fruits from each of 15 hand-thinned and 45 control plants.

TABLE 8. Effect of fruit-thinning on pulp and seed mineral content of *Lindera benzoin*, Fox Point, SERC, 1991.^a

	Pulp minerals		Seed minerals	
	Thinned	Control	Thinned	Control
N	4.916 (0.218)	4.845 (0.175)	24.141 (0.684)	23.015 (1.224)
P	0.811 (0.061)	0.773 (0.037)	3.176 (0.091)	2.921 (0.147)
K	19.784 (0.739)	18.522 (0.629)	4.779 (0.160)	4.661 (0.224)
Ca	0.322 (0.016)	0.306 (0.016)	0.761 (0.027)	0.764 (0.060)
Mg	0.380 (0.026)	0.342 (0.034)	1.308 (0.041)	1.186 (0.057)
Mn	0.005 (0.001)	0.006 (0.001)	0.037 (0.003)	0.038 (0.003)
Fe	0.009 (0.001)	0.010 (0.001)	0.041 (0.002)	0.038 (0.002)
Cu	0.002 (0.0002)	0.002 (0.0003)	0.023 (0.0006)	0.023 (0.0001)
B	0.006 (0.0002)	0.006 (0.0003)	0.006 (0.0002)	0.005 (0.0004)
Al	0.006 (0.0005)	0.006 (0.0004)	0.015 (0.0009)	0.014 (0.0011)
Zn	0.014 (0.0011)	0.014 (0.0009)	0.047 (0.0015)	0.042 (0.0020)
Na	0.133 (0.0076)	0.121 (0.0044)	0.015 (0.0013)	0.013 (0.0010)

^a Means, with standard errors in parentheses, for absolute content per fruit ($\times 10^{-4}$ g). Results obtained from samples of 50 fruits from each of 15 hand-thinned and 15 control plants.

DISCUSSION

Differential costs of reproduction—The results of this study suggest that females of the dioecious shrub *L. benzoin* bear relatively greater reproductive costs in terms of biomass and minerals; costs that are measurable in terms of future growth and reproduction. Estimates of biomass and nitrogen allocation to reproduction indicate much higher levels of investment by females, and individual ramets, terminal shoots, leaves, and lateral branches of female plants tend to grow more slowly than those of male plants. Analysis of annual incremental growth from stem cores taken from plants in other areas at SERC have shown relatively greater basal growth for males (M. Cipollini, unpublished data), which is consistent with the trend seen in this study.

Analyses at the whole plant level and at the level of reproductive modules indicate that flower number is primarily a function of plant or module size, and flower number/plant size relationships do not differ between males and females. Although flower mass was somewhat higher for male plants (mean = 0.0060 g per cluster for males and 0.0019 g per cluster for females in 1992), mineral element concentration was similar for flowers of both sexes (M. Cipollini, unpublished data). As with many dioecious species, it is the production of heavy, nitrogen-

rich fruits that results in greater reproductive allocation for female *L. benzoin*.

Females and males classified as newly flowering did not differ significantly in any size or growth variable in 1992 and 1993 (M. Cipollini, A. Mills, and D. Whigham, unpublished data). This suggests that the minimum size for flowering does not differ between the sexes, and that differential growth rates are seen only in reproductive plants. Although it is possible that adult regression to the non-flowering (juvenile) state may occur in *L. benzoin*, this has not happened in 3 census years at Fox Point. That is, all individuals flowering in 1991 flowered again in 1992 and 1993, and all 96 genets classified as newly flowering in 1992 flowered again in 1993. This suggests that a predominant number of individuals recorded as newly flowering did in fact flower for the first time, which is to be expected for a young, growing population (maximum population age is less than 40 years, D. Higman, SERC, personal communication). Taken together, these observations are consistent with the hypothesis that vegetative dimorphisms are a direct consequence of greater reproductive costs borne by adult females.

The lack of effects of fruit thinning on individual pulp and seed biomass and mineral allocation may be indicative of physiological or genetic constraints that may limit variation in allocation per flower or fruit (cf. Stephenson,

TABLE 9. Effect of fruit-thinning on growth and reproduction of *Lindera benzoin*, Fox Point, SERC, June 1991 to August 1992.^a

Parameter	Hand-thinned	Control	Main effect ^b	
			Treatment	Covariate
Basal diam increase (cm)	0.27 (0.04)	0.18 (0.04)	2.27	0.15
Height increase	36.83 (3.56)	25.84 (2.40)	0.12	5.74*
Volume increase (cm ³)	231.24 (36.4)	149.92 (28.7)	3.18	0.29
Fruit abortion 1991 ^c	0.04 (0.02)	0.10 (0.04)	2.83	2.67
Fruit set 1992 ^d	0.18 (0.03)	0.16 (0.02)	2.33	2.05
Ovule number 1992	7,956.00 (2,346)	4,301.13 (1,328)	3.78	12.92***
Fruit number 1992	1,029.13 (256.5)	535.67 (96.17)	8.99**	18.71****

^a Diameter, height, and volume increases are for the largest ramet in each genet; other growth and flowering data refer to the entire genet. Results are means and standard errors for 15 plants in each treatment.

^b *F*-values for results of separate ANCOVA analyses (main effect = fruit-thinning treatment). Initial size values were used as covariates for ANCOVA of growth parameters, and genet volume for ANCOVA of reproductive parameters.

^c Proportion of immature fruits that dropped during the period following hand-thinning (early June 1991) until fruit maturation in August 1991.

^d Proportion of ovules marked in April 1992 that subsequently developed into mature fruits by August 1992.

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$.

1981; Meagher, 1992). Additionally, rates of subsequent fruit abortion in 1991 were affected little by fruit thinning in June of that year, which suggests that fruit abortion rates in the latter part of the year are influenced little by resource availability. Fruit production was, however, significantly higher for hand-thinned plants in the following year (1992), indicating that reduced fruit levels in 1991 had lowered reproductive costs. This effect was not mediated through differences in plant growth, which was not significantly different between treated and control plants. These results support the hypothesis that high fruit set imposes costs in terms of future reproduction primarily through direct negative effects. Although we were unable to detect effects of hand-thinning on overall mineral allocation to seeds and fruit pulp, we did find differences in the patterns of mineral allocation in pulp and seed tissues. This implies that limitation of specific minerals has the potential to exert differential effects on allocation to seeds and pulp (seed/pulp ratios).

We propose that direct negative effects of prior fruit production are mediated through variation in levels of stored resources (e.g., starch, nitrogen, etc.; cf. Cipollini and Stiles, 1991; Newell, 1991), although we did not attempt to quantify variation in storage carbohydrates in vegetative tissues of these plants. The results of the hand-thinning experiment corroborate results obtained from the path analysis, both of which suggest direct negative effects of fruit crop size on subsequent fruit production. In the dioecious tree *Nyssa sylvatica*, direct negative effects of fruit production (i.e., reduction in carbohydrate and nitrogen levels) influences flower production in the subsequent year, such that female trees tend to flower only once every 2 years (Cipollini and Stiles, 1991). We were unable to detect strong direct effects of fruit production on flowering in *L. benzoin*, but rather found only direct effects on subsequent fruit production (which was a function of both flower number and fruit set).

Indirect effects of fruit production on subsequent flower production can also be mediated by negative effects on subsequent growth (cf. Newell, 1991); nevertheless, our experiment and path analysis do not support this hypothesis for *L. benzoin*. We suggest that the potential for increased subsequent growth in females with low fruit set in 1991 may have been ameliorated by higher levels of fruit production in these plants in 1992. These results illustrate the interdependency of resource level, growth, and reproductive output that makes detection of patterns under field conditions difficult without experimental manipulation (cf. Niesenbaum, 1993). Newell (1991) suggested that within-plant responses and physiological integration of plant modules may function to regulate reproductive costs such that substantive whole-plant growth responses are dampened. As such, a closer examination of within-plant variation in fruit production and modular growth is warranted for *L. benzoin*.

Dimorphism in carbon/nutrient balance—Our data show significantly higher levels of leaf phenolics, and trends for lower levels of shoot and leaf nitrogen for females of *L. benzoin*. These results provide some support for the carbon/nutrient hypothesis that predicts lower nitrogen and higher C-based secondary metabolites in females of di-

oecious plants (Jing and Coley, 1990). Because our total phenolic estimates were based upon a simple colorimetric assay, we must be somewhat cautious in interpretation of these data. Although we found no apparent differences in phenolic profiles between bulked shoot and leaf samples from each sex (as determined by silica gel TLC), differences among individual samples in the types of phenolics present could affect results of colorimetric tests such as the Prussian Blue or Folin-Denis assays (J. Schultz, Pennsylvania State University, personal communication). We encourage future tests of the carbon/nutrient balance hypothesis using dimorphic species such as *L. benzoin*, but emphasize that such tests should attempt to more rigorously quantify C-based secondary compounds. Ideally, effects of soil nutrient status and light level variation should be taken into account, especially in view of the considerable effect of light variation on growth and reproduction in *L. benzoin* (Veres and Pickett, 1982; Niesenbaum, 1992a, b, 1993).

Potential effects of environmental variation.—Variation in size and sex distribution patterns may occur if environmental variation differentially affects costs of reproduction for males and females, such that relative levels of growth and/or mortality vary among habitats. We observed a strongly female-biased adult sex ratio for *L. benzoin* in the Fox Point population. In two other populations of *L. benzoin* at SERC, as well as populations in West Virginia (1), Virginia (1), and Pennsylvania (2), we have observed sex ratios to differ as a function of plant size, such that smaller size classes (1 to 2 m in height) tend to be female-biased while the largest size classes (2+ m in height) tend to be male-biased (A. Mills, M. Cipollini, and D. Whigham, unpublished data). Overall adult sex ratios in these populations range from slightly male-biased to strongly female-biased (as in the Fox Point population). Because of the potential reduction in overall size as individual ramets within genets die and because of spatial and temporal variation in ramet growth and mortality, genet size may show little correlation with age. While relatively greater rates of growth in ramets of adult *L. benzoin* males can explain shifts in sex ratio with plant size, female-biased overall adult sex ratios cannot be explained by this factor alone. And, because the minimum size for flower production does not apparently differ between the sexes at Fox Point, differential mortality in small size classes falls out as the only likely ecological source of adult female-bias (assuming absence of sexual lability or biased sex ratios of seeds).

These observations have led to a two-sex matrix demographic model (cf. Meagher, 1982) of the Fox Point population that incorporates relatively greater levels of mortality for male seedlings and juveniles as a means of explaining observed sex ratio patterns (M. Cipollini, A. Mills, and D. Whigham, unpublished data). Despite differences in transition probabilities that explain shifts in sex ratio with plant size (e.g., greater likelihood of regression to the small size class and lower probability of growth to the large size class for females), overall sex ratios remain close to 1.0 under the assumption of identical transition probabilities for female and male seedlings and juveniles. Thus, we suggest differential mortality resulting

from differential herbivory of male and female seedlings and juveniles as an explanation for the female-biased sex ratio at Fox Point.

Although we know that herbivory by small mammals is a major source of seedling and juvenile mortality for *L. benzoin*, the hypothesis of differential mortality in these stage classes cannot be addressed directly because we have no means of determining the sex of individuals prior to first reproduction. Because plant quality for herbivores and the level of herbivory can vary as a function of habitat (cf. Elmqvist et al., 1988; Niesenbaum, 1992b), spatial variation in herbivory may be associated with direct and indirect effects on growth, mortality, costs of reproduction, and hence population demography.

In a comparison of adult male and female *L. benzoin* in Massachusetts, Primack (1985) reported no size differences and sex ratios that did not differ significantly from 1:1. While reporting somewhat greater growth in male plants, Niesenbaum (1992a, b) found sex ratios not significantly different from 1:1 in three "high-light" and three "low-light" habitats in southeastern Pennsylvania, and did not find evidence of sex differential herbivory by insects on adult plants. Despite extensive variation in levels of pollen reaching stigmatic surfaces, fruit set in these plants was influenced more by light level than by pollen level (Niesenbaum, 1992a, 1993), and fruit set was not elevated by artificial pollination (Niesenbaum, 1993). These observations are consistent with primarily resource-limited fruit production in *L. benzoin*. Niesenbaum (1992a) suggested that relative allocation to reproduction and growth (reproductive costs) in *L. benzoin* may depend upon the light environment, and females may thus suffer disproportionately greater costs under low light conditions. Although we have observed increases in growth, flowering, and fruit production of *L. benzoin* in response to forest canopy openings at SERC, the relationships of flower and fruit number to plant and shoot size did not differ between gap and understory plants (M. Cipollini, D. Wallace-Senft, and D. Whigham, unpublished data). That is, flower and fruit production were increased under high-light conditions in direct proportion to increases in new shoot growth. Additionally, we have observed no reduction in the fruit set of females that are potentially pollen-limited as a result of spatial isolation from males (i.e., 50+ m from nearest male; M. Cipollini, unpublished data). Taken together, these observations suggest that relative differential costs in terms of biomass and mineral allocation to reproduction may remain high across time and space for *L. benzoin*. That is, because of relatively consistent levels of fruit production, differences between the sexes in reproductive costs are likely to remain high, despite direct effects of prior fruit set and environmental variation that contribute to variation in fruit production.

Implications for lifetime costs of reproduction—In this study, we have demonstrated that estimates of biomass and nutrient allocation to reproduction differ strongly between males and females of the woody shrub *L. benzoin*. Sexual dimorphism was also manifested in differential patterns of growth and carbon/nutrient balance of ramets, leaves, and newly produced shoots of males and females.

As such, our data support the concept of cost of reproduction as it relates to within- and among-season trade-offs between vegetative and reproductive processes in dioecious perennials. Constraints on our ability to make more precise conclusions concerning lifetime reproductive costs relate to the complex life-form of *L. benzoin* that makes it difficult to estimate biomass and growth in the field, as well as the lack of information on belowground allocation, seasonal variation in tissue carbon and mineral balance (cf. Reekie and Bazzaz, 1987; Cipollini and Levey, 1992), and the potential interacting effects of herbivory and other sources of environmental variation. These are problems common to studies of long-lived woody perennials such as *L. benzoin*, and are factors that warrant future attention. In our study, we have shown that a negative feedback mechanism may act to regulate costs of reproduction in females of *L. benzoin*; there are direct negative effects of past reproduction on current reproduction. By making current reproduction dependent upon both current plant size and prior level of reproduction, this feedback process has potential consequences for population demography and lifetime costs of reproduction (cf. Calvo and Horvitz, 1990). We hypothesize that lifetime costs of reproduction should nevertheless differ strongly between males and females of this species, as a result of rather consistently high levels of fruit production across time and space. As a means of addressing this hypothesis, studies are needed to determine more precisely how spatial and temporal environmental variation influences demographic characteristics in this species. We are presently addressing this issue via a combined forest dynamics and patch-specific population dynamics matrix model analogous to that employed by Horvitz and Schemske (1986).

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