AN ABSTRACT OF THE THESIS OF

Stephen P. DiFazio for the degree of Master of Science in Botany and Plant Pathology presented on May 5, 1995. Title: The Reproductive Ecology of Pacific Yew (*Taxus brevifolia* Nutt.) Under a Range of Overstory Conditions in Western Oregon

Abstract approved Redacted for Privacy
Nan C. Vance Mark V. Wilson

The influence of overstory openness on the reproductive ecology of Pacific yew (*Taxus brevifolia* Nutt.) was investigated on 4 sites in western Oregon over 2 years. The breeding system of *T. brevifolia* was found to deviate from pure dioecy under a broad range of canopy and site conditions. Production of female strobili was observed on 17 of 58 predominantly male trees, while no male strobili were observed on 57 female trees. Genet sex ratios were significantly biased in only 1 population, where male genets outnumbered female genets by almost 2 to 1. Mean floral sex ratios were significantly male-biased in all populations and ranged from 5 to 12. Pollen-ovule ratios were in excess of 1,000,000 for all populations. In contrast, reproductive effort based on masses of mature strobili were female-biased by a factor of 1.1 to 5 for all sites. Seed masses also varied inversely with elevation.

Pollination phenology varied with elevation and overstory openness. Pollen first began shedding at the lowest sites, and earlier in trees under open conditions than in trees with overstory canopy cover. The duration of pollen shedding varied from 3 to 20 days, and tended to be

more protracted at lower sites and under open canopy conditions.

Most of the variation in reproductive potential, as indexed by strobilus production, occurred within sites and within trees. Little variation between years was observed in male strobilus production during the three years of this study. Also, while female strobilus production was significantly greater in 1993 than in 1994, seed production did not differ between years.

Overstory openness was positively associated with growth and reproductive potential of *T. brevifolia*. Specific leaf area was inversely correlated with overstory openness, and branching was positively correlated with overstory openness, suggesting that *T. brevifolia* adapts to overstory removal by producing denser foliar tissue and increased self-shading. In contrast to reproductive potential, seed production was not significantly associated with overstory openness during the two years of this study. Also, seed efficiency (the ratio of seed production to ovule production) was negatively associated with overstory openness.

Seed efficiency ranged from 5 to 34%, and attrition occurred in two phases. The early phase occurred during the pollination period and was probably due in part to pollination failure. Supplemental hand-pollination resulted in a doubling of seed efficiency on two of the sites, but average seed efficiency was still less than 15% on branches receiving supplemental pollen. Other potential sources of early attrition included damage from phytophagous mites, pathogens, frost and genetic incompatibility.

Attrition in the later stages of seed development was due in part to predation by vertebrate seed consumers. Predator exclusion significantly increased seed development efficiency (the ratio of seed production to developing ovules) on 3 of 4 sites over 2 years.

Seed production was positively correlated with overstory openness on branches bagged to exclude vertebrates, suggesting that resource availability was important for seed production in the absence of predation. However, evidence for resource limitation of seed production was not consistent. Seed efficiency was not significantly associated with overstory openness in 1993, and no associations were detected between vegetative growth or previous reproduction and seed efficiency in 1994.

Possible evolutionary explanations for low seed efficiency in *T. brevifolia* include the effects of sexual selection, stochasticity in pollination and predation, and the importance of excess ovaries as reserves that compensate for constant sources of mortality. Sources of seed attrition varied considerably among years and sites, emphasizing the importance of spatial and temporal variability in the reproductive ecology of *T. brevifolia*.

THE REPRODUCTIVE ECOLOGY OF PACIFIC YEW (*TAXUS BREVIFOLIA* NUTT.) UNDER A RANGE OF OVERSTORY CONDITIONS IN WESTERN OREGON

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CHAPTER 1 INTRODUCTION

LITERATURE REVIEW

Pacific yew (*Taxus brevifolia* Nutt.) is an understory conifer of the Pacific Northwest. Long disregarded by timber-conscious resource managers, *T. brevifolia* has become internationally famous as the original source for the anti-cancer drug taxol (Wani *et al.* 1971). More than half a million *T. brevifolia* trees have been destroyed since 1989 as a result of bark harvesting for taxol production (Anonymous 1993). Untold thousands of *T. brevifolia* trees have also perished as a result of decades of clearcut timber harvesting of old growth stands. The long-term effects of this mortality are not known. The research presented in this thesis was part of a larger effort to assess the impact of anthropogenic activities on the viability of wild *T. brevifolia* populations. The primary objective is to illuminate some poorly understood aspects of the reproductive biology of *T. brevifolia* through studies of populations in western Oregon. This introductory section will provide a general review of the current state of knowledge of the biology and ecology of *T. brevifolia* reproduction, and to provide an overview of the issues addressed in subsequent chapters.

Taxonomy and Evolution of the Genus *Taxus*

T. brevifolia is a member of the family Taxaceae, an ancient lineage dating back at least to the Triassic, nearly two hundred million years ago (Florin 1948). The earliest known representative of the genus is *Taxus jurassica* Florin, found in Jurassic sediments in England (Florin 1948).

The taxonomic position of the Taxaceae has been much debated during the past century. Florin (1948, 1954) argued for placing the Taxaceae in the class Taxopsida, apart from the Coniferopsida. This argument is based in part on the apparent lack of homology between the terminal, uniovulate strobilus of the Taxaceae and the compound, axillary cone scale complex of the 'true' conifers (Bierhorst 1971; Florin 1948, 1954). However, recent workers have argued that the Taxaceae share similarities in morphology and development with other conifers which are unlikely to have arisen independently (Miller 1988). Also, analyses of wood chemistry (Price 1990) and ribosomal DNA sequences (Chaw *et al.* 1993) do not support a separate class for the Taxaceae.

The taxonomy of the genus *Taxus* is as murky as that of the higher classifications. Estimates of the number of *Taxus* species range from 1 to 12 or more (Price 1990). The confusion is due in part to high phenotypic plasticity, high potential for interspecific hybridization, and morphological similarity of the various species. Most modern authors consider the genus to consist of 7 to 11 species, including *Taxus baccata* L. (European yew), *Taxus brevifolia* Nutt., *Taxus canadensis* Marsh. (Canada yew), *Taxus chinensis* (Pilger) Rehd. (Chinese yew), *Taxus cuspidata* Sieb. et Zucc. (Japanese yew), *Taxus floridana* Chapm. (Florida yew), *Taxus*

globosa Schlechtd. (Mexican yew), *Taxus Wallichiana* Zucc. (Himalayan yew), *Taxus maireii* (Lemee et Level.) S.Y. Hu, *Taxus celebica* (Warburg) Li, and/or *Taxus yunnanensis*. Recent efforts to differentiate the species have focused on leaf morphology (Spjut 1992), taxane chemistry, allozymes, and DNA restriction fragment length polymorphisms (RFLP) (Vance *et al.*, in preparation). These studies provide genetic evidence for recognizing at least 7 *Taxus* species.

There is a general lack of published information about the biology of *T. brevifolia*, but considerably more literature is available on other *Taxus* species, particularly those used in horticulture (e.g. Chadwick and Keen 1976; Krussmann 1983). Although *T. brevifolia* is morphologically and ecologically very similar to some of the other members of the genus, it is unclear how much of the information on other *Taxus* species also applies to *T. brevifolia*.

Distribution and Habitat

T. brevifolia occurs from southeast Alaska and British Columbia, along the Pacific coast into northern California, extending inland to the crest of the western Cascade mountains and the Sierra Nevada. It also occurs on the western slopes of the Rocky Mountains in northern Idaho and western Montana, and in scattered localities in eastern Washington and Oregon (Bolsinger and Jaramillo 1990).

T. brevifolia is primarily a late successional species, occurring most commonly in the understory of old-growth forests (Bolsinger and Jaramillo 1990; Busing *et al.* 1995; McCune and Allen 1985). An extensive survey of forest plots in western Oregon and Washington revealed that *T. brevifolia*

was most often associated with the late-successional species western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Busing *et al.* 1995). Despite this strong association, *T. brevifolia* is not an obligate understory species. In addition to old-growth forest associations, *T. brevifolia* occurs in all successional stages and in a wide range of soil conditions and vegetation associations throughout its range (Anonymous 1992a; Bolsinger and Jaramillo 1990; Busing *et al.* 1995). Although it seems to thrive best in a warm, maritime climate with a long growing season, *T. brevifolia* tolerates a wide range of temperatures and can be found from sea level to near timberline (Anonymous 1992a).

Although *T. brevifolia* commonly establishes beneath relatively intact forest canopy and is slow to recolonize after stand destruction (Busing *et al.* 1995; McCune and Allen 1985; Minore *et al.*, in preparation), establishment does occur in open conditions (Betlejewski 1993; Minore *et al.*, in preparation). Overstory removal by timber harvest does not normally prove fatal to *T. brevifolia* (Crawford 1983; Bolsinger and Jaramillo 1990), and open-grown *T. brevifolia* sometimes displays greater growth and seed production than understory trees (Bailey and Liegel in preparation; Chapter 3). Apparently thriving *T. brevifolia* trees have been observed in such uncharacteristic habitats as avalanche chutes (Anonymous 1992a) and basalt lava flows (personal observation).

Despite its wide range of adaptability and ecological tolerances, *T. brevifolia* occurs only sporadically in most of its range, primarily as an understory species of old growth forest. The most commonly cited limits to *T.*

brevifolia occurrence are sensitivity to fire and inefficient colonization of available habitat (Busing *et al.* 1995; McCune and Allen 1985). Fire normally proves fatal to thin-barked *T. brevifolia* (Crawford 1983; Stickney 1980), and this partly explains the strong association of *T. brevifolia* with other fire-sensitive, shade-tolerant, late seral species such as *T. heterophylla* or Port-Orford-cedar (*Chamaecyparis lawsoniana* (A.Murr.)Parl.) (Busing *et al.* 1995; McCune and Allen 1985; Scher and Jimerson 1989). Inefficient seed dispersal and/or establishment following major disturbance may contribute to the relative lack of *T. brevifolia* in early successional stands dominated by Douglas-fir (*Pseudotsuga menziesii* (Mirb.)Franco) (Busing *et al.* 1995; McCune and Allen 1985).

Population Structure

Surveys of the genetic structure of *T. brevifolia* populations have been carried out throughout most of the species' range, and the results provide insight into the reproductive biology of the species. *T. brevifolia* exhibits moderate diversity of allozyme alleles, averaging 42% polymorphic loci, 1.5 to 1.7 alleles per locus and expected heterozygosity values of 0.13 to 0.17. Variation is primarily partitioned within populations, with only 8 to 10.7 percent of the observed variation occurring among populations (Doede *et al.* 1993; El-Kassaby and Yanchuk 1994; Wheeler *et al.* 1993). These values are typical for woody perennials with restricted ranges (Anonymous 1993; Loveless and Hamrick 1984). In addition, observed heterozygosity in British Columbia was significantly lower than expected, and

the inbreeding coefficient was comparable to that of other species with both sexual and asexual reproduction (El-Kassaby and Yanchuk 1994). This inbreeding could be due in part to pronounced clustering of related individuals within populations. Evidence of such population structure in Oregon and Washington was provided by analyses that revealed that most within-population variation in allozymes occurred among locales within the population (Doede *et al.* 1993).

The low genetic variation exhibited among widely scattered *T. brevifolia* populations suggests that gene flow among populations is high, or that all sampled populations were derived from a single small population, and migration was relatively recent. In either case, considerable vagility relative to generation time is implied (Loveless and Hamrick 1984).

El-Kassaby and Yanchuk (1994) speculate that the major mechanism of gene flow for *T. brevifolia* is likely to be long-distance dispersal of seed by birds. The authors expect that gene flow through pollen is likely to be somewhat restricted due to the understory habit of *T. brevifolia*. The efficiency of gene transfer by seed should be greater than that of pollen because seeds carry twice as many genes from the source population. Also, a seed has a greater chance of becoming established in a foreign population than a pollen grain because the seed has one less selective phase to pass through (Loveless and Hamrick 1984). It is also possible that birds disperse seeds over much greater distances than wind-dispersed pollen. However, there is no evidence that pollen flow is less than seed flow

in *T. brevifolia*, and pollen could travel long distances if caught in turbulent eddies (Whitehead 1983).

Sexual Reproduction

Breeding System

With the exception of *T. canadensis*, all members of the Taxaceae are dioecious, bearing male and female reproductive structures on separate genets. The mode of sex determination in *Taxus* is unknown. The haploid chromosome number appears to be 12, and there are no obvious sex chromosomes (Chadwick and Keen 1976; Dark 1932). There is evidence that *Taxus* sex expression is somewhat plastic and may be partly environmentally determined (Allison 1991; Freeman *et al.* 1980). Sex reversal has been observed in young plants of several *Taxus* species (Chadwick and Keen 1976), and ovules have been observed on male genets in *T. baccata*, *T. cuspidata* and various cultivars (Chadwick and Keen 1976; Pridnya 1984). Also, *T. canadensis* is primarily monoecious but also exhibits gender dimorphism, perhaps in response to environmental cues such as resource levels or grazing by deer (Allison 1991, 1992).

Morphology of Male Strobili

Taxus male strobili occur primarily on the underside of branches in the axils of leaves of the current year's growth (Price 1990). Bud differentiation typically occurs in the summer of the previous growing season (Hejnowicz 1975), and the globose developing strobili are distinguishable from vegetative buds in July in western Oregon (personal observation). Mature strobili consist of 6-14 radially

arranged, peltate microsporophylls (stamens) distal to tightly clustered, sterile scales. Each microsporophyll bears 6-10 microsporangia (pollen sacs) at its apex. The pollen is held in the microsporangium by stiff membranous flaps. One end of the flap is attached to the axis of the microsporophyll, while the other end curls under the microsporangial base. As the flaps dry they curl upward, releasing the pollen to the air.

Pollen grains measure about 25 microns in diameter. They are globose, nonsaccate, and lack prothalial cells (Florin 1948). The grains are surrounded by a 2-layer non-porous exine, an intermediate layer, and an intine. The intermediate layer swells upon wetting, and is potentially important in pollen germination (Hejnowicz 1975).

Morphology of Female Strobili

The female strobilus of *Taxus* consists of a terminal, atropic ovule subtended by 3 pairs of decussate scales (Price 1990). The whole structure can be considered homologous to the strobili of other conifer groups. The solitary, terminal ovule may have evolved by loss of all distal parts, and the subtending scales may be homologues of cone bracts (Miller 1988). Female strobili usually occur on the underside of branches on the current year's growth, but exceptions are common (personal observation). Differentiation occurs during the summer of the previous season (Hejnowicz 1975), but female strobili are difficult to distinguish from vegetative buds with the naked eye until after anthesis. The ovule is borne on a very short, second-order shoot. The primary shoot originates in the axil of a

vegetative leaf. The primary shoot sometimes terminates in a bud which can be either vegetative or reproductive. Under some circumstances the terminal buds of both the first and second order shoots may develop, resulting in apparently twinned ovules (Bierhorst 1971; Niklas 1985; personal observation). Occasionally, the terminal bud may remain dormant over winter and undergo development the following year (Allison, personal communication; personal observation).

Pollination

At anthesis the apex of the ovule emerges from beneath the basal scales. A viscous pollination drop consisting of complex carbohydrates and amino acids is secreted by the cells of the nucellus (Seridi-Benkaddour and Chesnoy 1988). This drop captures pollen grains and draws them into the pollen chamber. The presence of the pollination drop on the micropyle of the ovule is probably the best indicator of female receptivity. Pollination drops of *T. brevifolia* can persist from several hours to several days (personal observation).

Pollination in *Taxus* is by wind, and typically occurs early in the growing season, during March and April for *T. brevifolia* in Oregon (Chapter 2). The efficacy of pollination can depend on weather conditions at the time of pollen shed (Anonymous 1992a; Chadwick and Keen 1976), synchrony in male and female phenology (Pridnya 1984) and the density, proximity and fecundity of male trees in the population (Chapter 4; Allison 1990b).

Niklas (1985) studied the aerodynamics of *Taxus* pollination using a wind tunnel and a scale model of a *T. cuspidata* ovule. The structure of the ovule is such that nearby air currents are directed toward the micropyle and the leeward surface of the ovule. Niklas (1985) also hypothesized that the pendant ovules of *Taxus* serve as drip points and that rain water carries pollen from proximal portions of the branch and ovule to the micropyle. This would greatly improve the efficiency of pollination by reducing the necessity for direct impact of pollen grains with the micropyle and strict concordance of male and female phenology. Precipitation events are frequent during the pollination period for *T. brevifolia* in the Pacific Northwest, and such a pollination mechanism would greatly improve pollination efficiency. However, there is presently no direct evidence that water-mediated pollination occurs in wild *T. brevifolia* populations.

Ovule and Seed Development

Ovule development in *Taxus* ensues shortly after pollination; unpollinated ovules do not develop (Allison 1990b; Chapter 4). Fertilization occurs 6-12 weeks after pollination (Hejnowicz 1975). The ovule expands and the micropyle closes. A fleshy aril develops as the margins of a transverse plate of meristem subtending the ovule begin to swell (Bierhorst 1971). The aril slowly covers the expanding seed as the season progresses. The mature aril is scarlet and cuplike; it nearly surrounds the seed but is only basally adnate. The basal scales expand into a receptacle which remains attached to the branch for several

days following dehiscence or removal of the ripe seed. The development of the aril can vary markedly, and the aril sometimes fails to fully form (Chadwick and Keen 1976; personal observation). The condition of the aril may be an indicator of seed viability, as seeds with malformed arils have shown lower germination rates than seeds with normal arils (Heit 1969).

Seed Dispersal

The seed of *Taxus* is well-adapted for dispersal by birds and mammals. The bright red color of the aril is a strong cue for many bird dispersers (Howe and Westley 1986). The thick seed integument survives passage through bird digestive systems, resulting in long-distance dispersal (Sakakibara 1989; Suszka 1975). In fact, germination may even be enhanced by vertebrate processing of *Taxus* seed (Rudolph 1974). The presence of cyanogenic glycosides in the seed integument, which are converted to hydrocyanic acid when the tissue is disrupted, may provide some protection against seed predation. However, the aril and contents of the seed are not toxic (Barnea *et al.* 1993), and a wide variety of birds and mammals feed on these without digesting the toxic integument.

Most of the information about animal removal of *Taxus* seed is based on sporadic or anecdotal observations, with few intensive studies. A large variety of animals have been observed feeding on the seed of *T. baccata* in Europe, including fieldfares, redwings, thrushes, blackbirds, nuthatches, finches, pheasants, mice, voles badgers and foxes (Smal and Fairley 1980; Suszka 1975; Tittensor 1980).

Crawford (1983) reports that gray jays (*Perisoreus canadensis*), Steller's jays (*Cyanocitta stelleri*), varied thrushes (*Ixoreus naevius*), western tanagers (*Piranga ludoviciana*), squirrels and chipmunks take *T. brevifolia* seeds in Idaho (bird nomenclature after Peterson 1990). Pilz (unpublished) reports finding mouse caches of *Taxus* seed.

Sakakibara (1989) exhaustively characterized the fates of seeds from two *T. cuspidata* trees in Japan. More than 80% of the seeds produced by these trees were removed by birds and mammals, including tits, jays, thrushes and rodents. Although the vast majority of the seed was apparently destroyed by destructive feeding, scatter hoarding by the varied tit (*Parus varius*) appeared to be largely responsible for most of the existing seedlings in the stand. Also, a small percentage of the seeds were carried off by birds that swallowed them whole.

Observations of Seed Removal in Western Oregon

In three field seasons in western Oregon, seed removal by Townsend's chipmunks (*Tamias townsendii*), red-breasted nuthatches (*Sitta canadensis*), black-capped chickadees (*Parus atricapillus*) and gray jays was observed by the author. Dark-eyed juncos (*Junco hyemalis*), varied thrushes, Steller's jays, sparrows, house wrens (*Troglodytes*), yellow-bellied sapsuckers (*Sphyrapicus varius*) and golden-crowned kinglets (*Regulus satrapa*) have also been observed foraging in *T. brevifolia* trees, but seed removal by these species was not observed.

The mode of foraging for *T. brevifolia* seed in Oregon differs in important ways. Townsend's chipmunks feed extensively on immature seed, and appear to process most seed *in situ*. They hang from the underside of branch tips, bite holes in the integuments and remove the seed contents. Discarded integuments with bite holes are commonly found under productive *T. brevifolia* trees. Townsend's chipmunks have also been observed removing ripe seed from the arils and dropping the seed at the base of the tree. This behavior may indicate caching of *T. brevifolia* seed by chipmunks (Crawford 1983), which could provide an important mode of seed dispersal (Sakakibara 1989). However, it appears that Townsend's chipmunks are primarily predators of *T. brevifolia* seed in western Oregon (personal observation).

Red-breasted nuthatches typically forage by flying repeatedly from *T. brevifolia* trees to perches up to several hundred meters away. Seed processing by nuthatches was not directly observed. It is possible that they engage in caching behavior like European nuthatch species (Crawford 1983). If so, nuthatches are potentially good short-distance seed dispersers, much like the varied tit in Japan (Sakakibara 1989).

Gray jays do not appear to feed destructively. They sometimes swallow the seed and aril whole, or they may immobilize the sticky seed on a tree limb and remove most of the aril, leaving the intact seed affixed to the bark of the tree (personal observation). The most effective dispersers of *Taxus* seed are probably birds that swallow seeds whole, fly away, and void viable seed at some distance. Mortality of seeds falling in the vicinity of the parent tree could be

extremely high due to increased levels of predation and interspecific competition (Howe 1977). The available evidence suggests that jays and other endozoochoric birds play an important role in the life history of *T. brevifolia*.

Seed Banks

An unknown proportion of *T. brevifolia* seeds are dispersed by gravity and persist in the vicinity of the tree as a seed bank. A study of soil seed banks around several isolated *T. brevifolia* trees in western Oregon indicated that an average of only 16 intact seeds per square meter occurred beneath the crown of the trees, and the quantity of seed declined markedly with distance from the tree (Minore *et al.* in preparation). In a related study (Minore *et al.* in preparation), seedlings were measured and aged around *T. brevifolia* stumps in a fenced plot that had been clear-cut and burned 7 years before. The seedling ages indicated that recruitment from the soil seed bank occurred continuously for 6 years following destruction of the parent trees, peaking 2 years after the fire and tapering off in subsequent years. These results suggest that *T. brevifolia* forms persistent seed banks that can be a significant source of recruitment following a major disturbance.

Germination

Most *Taxus* seeds require two full winters in the soil before they germinate (Rudolph 1974). Germination may be hastened somewhat by warm-cold stratification, but most seeds remain dormant for at least twelve months (Heit 1969; Pilz unpublished; Rudolph 1974). Excised embryos can be induced to germinate *in vitro* within weeks (Chee 1994;

Flores *et al.* 1993), which suggests that dormancy may be due in large part to interactions between the embryo and the megagametophyte. For a more complete discussion of germination requirements see Rudolph (1974) and Suszka (1975).

Vegetative Regeneration

Vegetative regeneration (or clonal growth, Harper 1977) is extremely common in *Taxus*. Genets spread by layering when limbs or boles are pressed to the ground by fallen trees or snow and adventitious roots form. *Taxus* may also coppice from stumps or root stocks when the main bole is cut (Bolsinger and Jaramillo 1990). *Taxus* exhibits a predominantly passive cloning habit, reacting to exogenous disturbance such as falling debris from the overstory to initiate clonal growth (Redmond 1984). It is possible that the thin cambium of *Taxus* is sensitive to sun scorching, which suggests that sprouting should be more vigorous under intact canopy (Anonymous 1992a; Crawford 1983). However, an extensive study of the occurrence of sprouting from stumps of harvested trees revealed few strong environmental determinants of sprouting. The number of stump sprouts prior to harvest was the most descriptive factor measured, being positively related to occurrence and survival of sprouts after harvest (Minore, in review). Sprouting appears to be rare following fire (Crawford 1983; Minore 1993), but there are reports of root stocks sprouting following light burns (Betlejewski 1993; Bolsinger and Jaramillo 1990; Busing *et al.* 1995).

There is some evidence that the ability of *T. brevifolia* to form adventitious roots is under genetic control. Vegetative cuttings show marked variation among clones in rooting efficiency under controlled conditions (Doede *et al.* 1993; Mitchell 1995; Vance, unpublished data). If the ability to form roots from cuttings is related to ability to layer or sprout, then this may indicate that vegetative regeneration is under genetic control. However, age of source trees, a factor in rootability, was not accounted for in the above studies, so evidence for genetic control of rooting efficiency is not complete.

Establishment and Survival

Natural populations of *T. baccata* are apparently on the verge of extinction in many parts of Europe, partly due to low levels of regeneration (Krol 1975; Melzack and Watts 1982; Mitchell 1990; Paule and Gomory 1993). Grazing by ungulates is the most commonly cited reason for lack of *Taxus* regeneration. Although *Taxus* foliage is highly poisonous to livestock (e.g. Panter *et al.* 1993), wild mammals such as deer, elk, moose, and rabbits feed heavily on *Taxus* foliage and bark (Crawford 1983; Krol 1975; Tittensor 1980). Herbivory is cited as being a major source of mortality for *Taxus* seedlings and sprouts in Iran (Mossadegh 1993), Poland (Krol 1975), England (Tittensor 1980), and Ireland (Mitchell 1990). A chronosequence of *T. baccata* succession in England indicated that *T. baccata* abundance increased as grazing animals were excluded due to changes in land use and the growth of protective juniper and hawthorn scrub (Tittensor 1980). The distribution of *T.*

canadensis in Wisconsin and Michigan is negatively correlated with the intensity of deer browsing (Allison 1991; Waller and Alverson 1993).

Other factors that may increase the establishment and survival of *Taxus* seedlings include the amount of litter or organic matter present in the soil (Crawford 1983; Krol 1975), water availability, and openings in the overstory canopy (Govil 1993; McCune and Allen 1985). It has also been hypothesized that *Taxus* litter exerts allelopathic effects on *Taxus* seedlings (Krol 1975), so that regeneration beneath *Taxus* canopy is relatively rare. In fact, few seedlings have been observed in western Oregon in the vicinity of existing *T. brevifolia* trees (Liegel and Bailey, in preparation; personal observation).

CONCLUSIONS AND OVERVIEW OF THESIS

Most of the available information about the ecology and life history of *T. brevifolia* suggests that seed production and dispersal are important in determining the distribution and persistence of *T. brevifolia* populations. *T. brevifolia* is highly susceptible to destruction by fire, and seed banks in the soil and/or seed immigration are probably the main modes of recolonization following a stand-replacing fire. *T. brevifolia* populations are also subject to a variety of anthropogenic disturbances throughout the range of the species, including the direct effects of bark harvesting and the indirect effects of harvesting of overstory trees. Vegetative regeneration following destruction by harvesting activities may be only sporadically and locally effective. Therefore, it is important to identify factors associated

with *T. brevifolia* seed production in order to predict the impact of anthropogenic activities on the viability of *T. brevifolia* populations.

Information on *T. brevifolia* reproductive biology is evolutionarily significant as well. The patterns of sex expression, sex ratios and sex allocation are relevant to the evolution and maintenance of dioecy in the Taxaceae. In addition, identification of the ecological factors that influence seed production can shed light on the evolutionary significance of patterns of seed production and seed attrition.

This thesis contains three main chapters, each of which addresses different aspects of the reproductive biology of *T. brevifolia*. Chapter 2 describes general patterns of sex expression, sex ratios, phenology and seed production. Previously undocumented information about *T. brevifolia* reproductive biology is presented in this chapter. Chapter 3 is an examination of the relationship between overstory canopy openness and the reproductive potential and growth of *T. brevifolia* trees. Chapter 4 provides analyses of the mechanisms of ovule and seed attrition in relation to overstory openness. Where appropriate, the evolutionary implications of the results reported in this thesis are explored. The overall significance of the work is summarized in a concluding chapter.

CHAPTER 2 VARIATION IN THE REPRODUCTIVE BIOLOGY OF TAXUS BREVIFOLIA IN WESTERN OREGON

INTRODUCTION

Available information about the ecology and life history of *T. brevifolia* and other *Taxus* species suggests that seed production and dispersal are important in determining the distribution and persistence of *Taxus* populations (Busing *et al.* 1994). While vegetative regeneration is probably effective at maintaining and expanding local populations, seed dispersal is essential for colonizing new sites. Also, given the high sensitivity of *T. brevifolia* to fire, which occurs often throughout most of the species' range, seeds and seed banks take on an added importance as potential sources of reestablishment following stand destruction.

The objective of this chapter is to present data on key aspects of *T. brevifolia* sexual reproduction in order to provide insight into the ecological and evolutionary dynamics of the species. The principal questions addressed are the following: What are the patterns of sex expression in *T. brevifolia* populations? Do sex ratios vary from unity? How do genet sex ratios, floral sex ratios and pollen-ovule ratios vary among populations? Does phenology vary with elevation and canopy closure? What are the patterns of seed maturation? How does seed production vary within and among years? Answers to such questions will deepen understanding of some of the ecological and evolutionary factors that shape *T. brevifolia* reproduction.

METHODS

Study Design and Tree Selection

This study was conducted on 4 sites in western Oregon (Table 2.1). The main criteria for site selection were the presence of at least 10 sexually mature *T. brevifolia* trees per hectare growing under a range of overstory canopy cover. Working systematically from an arbitrary starting point, all *T. brevifolia* trees greater than 3 m tall and 5 cm basal diameter were mapped until at least 30 male and 30 female trees were included. Trees separated by 2 m or less were considered ramets of the same genet, and were mapped as one tree. None of the trees selected for intensive study were within 3 m of one another.

In order to ensure an adequate range of overstory canopy cover, trees were selected by stratified random sampling. Trees were subjectively assigned to two groups: those growing under open canopy (OPN) and those growing under moderate to heavy canopy (CAN). These subjective designations were later quantified (Table 2.1; see below). Seven male and seven female trees were randomly selected from each canopy grouping, for a total of 28 trees per site. This selection technique resulted in two spatially separated populations per site, as described below. In this thesis, 'site' will refer to entire drainages, and 'plot' will refer to canopy cover groupings within a site.

Table 2.1 Sites used in the study. HC1=Higher Cascade 1; HC2=Higher Cascade 2; LC=Lower Cascade; VAL=Willamette Valley. N- number of trees measured; Values in parentheses are 1 standard deviation.

Site	Canopy Group	Elev. (m)	Vegetation Association[1]	N	DIFN[2,3]	Slope (degrees)[3]	Aspect (degrees)[3]	Tree Age[3]	Precip. (mm)[4]
HC1	OPN	1100	TSHE/RHMA/GASH	14	0.91(0.02)	27(5)	264(20)	87(41)	2569
	CAN	1200	TSHE/BENE/ACTR	14	0.21(0.07)	27(5)	192(29)	122(45)	
HC2	OPN	1150	ABAM/BENE	16	0.37(0.24)	14(4)	40(22)	153(44)	2143
	CAN	1035	ABAM/RHMA/XETE	14	0.11(0.06)	8(3)	167(161)	141(36)	
LC	OPN	850	TSHE/RHMA-BENE	14	0.47(0.18)	11(7)	192(29)	151(40)	2098
	CAN	850	TSHE/BENE/ACTR	14	0.16(0.10)	8(3)	211(46)	132(38)	
VAL	OPN	200	ABGR/RUUR-RHDI	15	0.83(0.09)	19(4)	206(60)	88(21)	1094
	CAN	300	ABGR/BRSY ABGR/DIHO-THOC	14	0.16(0.12)	19	67(40)	78(18)	

[1]See Hemstrom *et al.* (1987) for HC1, HC2 and LC; Hubbard (1991) for VAL.
[2]Diffuse noninterceptance, an estimate of the amount of sky visible from beneath the canopy. DIFN ranges from 0 (completely closed canopy) to 1 (no canopy).
[3]Population mean of values measured at individual trees.
[4]Average annual precipitation values derived from interpolations of 10 year averages using the PRISM climate model (Daly *et al.* 1994).

Study Sites

Higher Cascade 1 (HC1)

This site was located between Burnside Road and U.S. Highway 20 in the Snow Creek drainage of the Willamette National Forest (44° 23' 30" N; 120° 95' W). The open canopy plot (OPN) was located in a clearcut at an average elevation of about 1100 m. The slopes averaged 27°, and the aspect was primarily west to southwest. The dominant vegetation association was *Tsuga heterophylla/Rhododendron macrophyllum/Gaultheria shallon* (TSHE/RHMA/GASH)[1], indicating warm, dry, conditions for the TSHE zone (Hemstrom *et al.* 1987). The overstory trees were harvested in 1990, and the plot was not burned. There was substantial natural conifer regeneration occurring at the time of this study, including *Tsuga heterophylla*, *Thuja plicata*, and *Pseudotsuga menziesii*[2]. The sampled population occupied approximately 2 ha.

The overstory canopy plot (CAN) was located approximately 1 km upslope of the clearcut at an average elevation of about 1200 m. The slopes were comparable to those of the clearcut, and the aspect was south to southwest. The plot was primarily of the *Tsuga heterophylla/Berberis nervosa/Achlys triphyllum* vegetation association (TSHE/BENE/ACTR). This association is typical of relatively dry and cool sites within the *Tsuga*

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[1]Because this vegetation association was characterized at an early successional stage, it may not accurately represent the true late seral association.

[2]Plant nomenclature follows Hitchcock and Cronquist (1976).

heterophylla zone (Hemstrom *et al.* 1987). The overstory consisted primarily of *Pseudotsuga menziesii* and *Tsuga heterophylla*, with increasing amounts of *Abies amablis* in the upper portions of the plot. The overstory canopy was fairly continuous, though a substantial number of large trees had been blown down over the years. The *T. brevifolia* population occupied approximately 20 ha.

The soils of the site were primarily of the Willamette National Forest Soil Resource Inventory (SRI) mapping unit number 235 (Legard and Meyer 1973). This formation is characterized by deep soils derived from colluvium and residuum. The topsoil is a thin loam and the subsoil is clay loam, silty clay loam and clay. The parent material is primarily pyroclastic material, including breccias and tuffs (Legard and Meyer 1973).

This site had the highest average annual precipitation of the four sites over the previous ten years, 2564 mm. A persistent snow pack formed during winters of 1993 and 1994.

Higher Cascade 2 (HC2)

This site was located in the Hackleman Creek drainage of the Willamette National Forest (44° 24' N; 122° 02' W). The CAN plot was located at approximately 1035 M elevation on the east side of Forest Road (FR) 2672. The slopes on this plot were gentle, averaging 8°, and the aspect was generally north to northeast. The plot was bisected by a seasonal stream. The vegetation association was primarily *Abies amablis/Rhododendron macrophyllum/ Xerophyllum tenax* (ABAM/RHMA/XETE). This association indicates a warm, moist environment for the ABAM zone, and perhaps low soil

nitrogen. It is generally characterized by low productivity of the overstory trees (Hemstrom *et al.* 1987). The overstory mainly consisted of *Pseudotsuga menziesii, Abies amablis, Tsuga heterophylla* and *Pinus monticola.* The overstory was patchy due to the presence of large numbers of dead standing and windthrown trees. The sampled population occupied approximately 2 ha.

The OPN plot was located approximately 1.5 km upslope of the CAN plot, north of FR 410, at an average elevation of about 1150 m. The predominant vegetation association was *Abies amablis/Berberis nervosa* (ABAM/BENE), with scattered sectors of ABAM/RHMA/XETE. The ABAM/BENE association is indicative of a cool, well-drained site that has somewhat moister conditions than the ABAM/RHMA/XETE association (Hemstrom *et al.* 1987). The overstory consisted primarily of *Pseudotsuga menziesii* in this portion of the site. The stand was thinned in 1978, at which time all of the understory *Tsuga heterophylla* and *Abies grandis* were removed. In 1982, the slash was piled and burned on the perimeter of the plot. The overstory ranged from partially open to almost completely open on the more exposed portions of the site, due to differences in the intensity of windthrow.

The site had deep, well-drained soils primarily of SRI mapping unit 66 type (Legard and Meyer 1973). These soils have a thin, sandy loam topsoil and a thick and gravelly subsoil. The bedrock consists of andesites and basalts.

This site received 2143 mm of precipitation on average over the previous 10 years, and a snow pack persisted through most of the winters of 1993 and 1994.

Lower Cascade (LC)

This site was located in the McCrae Creek drainage of the H.J. Andrews Experimental Forest along FR 320 (44° 15' N; 122° 11' W). The CAN plot was located at about 850 m average elevation. The topography consisted of mild slopes, averaging 14°, with north to northeast aspects. The major vegetation association was *Tsuga heterophylla/ Rhododendron macrophyllum-Berberis nervosa* (TSHE/RHMA-BENE). This association is usually characteristic of cool, dry, productive sites for the TSHE zone (Hemstrom *et al.* 1987). The overstory consisted primarily of *Pseudotsuga menziesii*, *Tsuga heterophylla* and *Thuja plicata*. There was a substantial amount of blowdown in the stand, and the overstory canopy was somewhat uneven. The sampled population occupied approximately 2 ha.

The OPN plot was diverse and situated along the edge of several timber harvest units and along FR 320 at an average elevation of about 850 m. The slopes were quite variable, ranging from 1 to 22°. Aspects ranged from southeast to due west. The dominant vegetation association was TSHE/BENE/ACTR. The *T. brevifolia* population was mostly spread out linearly along FR 320. The sampled population occupied approximately 10 ha in two disjunct sectors. The overstory ranged from sparse to completely absent, and was mainly composed of *Tsuga heterophylla*, *Pseudotsuga menziesii* and *Thuja plicata*. The range in overstory canopies was due primarily to the diverse silvicultural history of the plot. A portion of the population occurred on the edge of a harvest unit that was clearcut and broadcast burned in 1981. Other sectors of the population were affected by a salvage

harvest in 1979 in which dead and diseased trees were removed. The primary source of overstory gap for most of the trees was Forest Road 320, which was constructed more than 35 years before this study was initiated.

The soils of the site were primarily of the SRI mapping unit 23 (Legard and Meyer 1973), similar to those described for site HC1.

The site received an average of 2098 mm of annual precipitation over the previous 10 years. A snow pack persisted through most of the winter of 1993 and part of 1994.

Willamette Valley (VAL)

This site was located on the western margin of the Willamette Valley in the foothills of the Oregon Coast Range. It was in the Oak Creek drainage of McDonald Research Forest of Oregon State University (44° 24' N; 122° 02' W). The CAN plot was located along both sides of FR 6020, with an average elevation of approximately 200 m. The slopes were relatively steep, averaging 19°, with aspects primarily ranging from due north to due east. In the upslope portions of the site the primary vegetation association was *Abies grandis/Brachypodium sylvaticum* (ABGR/BRSY) (Hubbard 1991) while in moister areas closer to the creek, vegetation mainly formed the *Abies grandis/ Disporum hookeri-Thalictrum occidentale* (ABGR/DIHO-THOC) association. The ABGR/BRSY association occurs at the dry end of an environmental moisture gradient in McDonald Forest, while the ABGR/DIHO-THOC association indicates moist to intermediate conditions (Leavell 1991). The overstory was composed of *Pseudotsuga*

menziesii, *Abies grandis* and *Acer macrophyllum*. Parts of the stand were harvested between 1915 and 1920, and a thinning operation took place in 1967. Also, at the time of this study several of the *T. brevifolia* trees were influenced by the gap formed by road 6020, which was constructed in 1963. The sampled population occupied approximately 10 ha.

The OPN plot was located approximately 2 km away from the CAN plot at an average elevation of approximately 350 m. The slopes were much like those of the CAN plot, and the aspect was primarily south to southwest. The main vegetation association was the *Abies grandis/Rubus ursinus-Rhus diversiloba* (ABGR/RUUR-RHDI) type (Hubbard 1991)[1], which indicates dry site conditions for the ABGR zone (Leavell 1991). The overstory trees were clearcut harvested in 1984 and the stand was planted with *P. menziesii* seedlings. The seedlings were hand-fertilized and competing vegetation was sprayed with herbicide in 1986. The sampled population occupied approximately 5 ha.

Annual precipitation averaged 1094 mm, and persistent snow cover was relatively rare. Snow persisted for about 2 weeks in 1993, and was negligible in 1994.

Male Strobilus Production

Strobilus production was determined on subsampled branches, and no attempt was made to estimate the productivity of entire trees. This method should reveal trends that might otherwise have been obscured by errors in estimates of whole tree productivity.

A pilot study was performed in 1992 on a subset of the trees used in 1993 and 1994. Ten branches were selected on each tree, clipped, and strobili were counted in the lab.

In 1993 8 branch tips were selected on each tree by stratified random sampling. Each tree crown was first divided into upper and lower sectors and 4 branches were randomly selected from each sector. Beginning at the basipetal portion of the branch, successive bifurcations were randomly chosen until arriving at a branch apex. Branches were measured 20 cm basipetal from the selected apex and marked with nylon flagging. All branch tips were sampled acropetal to the 20 cm mark. Strobili were counted in April and categorized as 'viable' and 'nonviable' based on color, external morphology and size. These designations were confirmed by following the fates of individual strobili.

In April of 1994 new branch apices were selected to compensate for mortality (≈1% of all branches). Each branch was clipped at the 1993 flagging and transported to the lab in a paper bag. Each branch sample was remeasured 20 cm basipetal from the apex, clipped, and all branch material between 20 cm and the 1993 flagging was discarded. The number of strobili on these 20 cm branch tips were counted and categorized as 'viable' and 'nonviable' as described previously. Stems and foliage were weighed after drying at 65° C for 48 hours.

Strobilus Mass and Pollen Production

The number of pollen grains produced per strobilus was approximated by multiplying the average mass of pollen

contained in mature strobili by the average number of pollen grains per gram. Strobili used in determining the mass of pollen were from branches collected in April 1994 as described previously. 10 mature strobili containing pollen and 10 strobili that had shed all pollen were collected from 2 randomly selected trees per site. Strobili were dried at 65° C for 48 hours and weighed individually to the nearest 0.00001 g. Average mass of pollen per strobilus was estimated from the difference in average mass of strobili that had shed pollen and strobili that had not shed.

Pollen was collected by clipping branches in March and April of 1994 from at least five trees at each site. Cut branch ends were placed in water at room temperature overnight and pollen was collected on waxed paper. Pollen was stored in corked glass vials at 4°C. Pollen from each site was dried at 65°C for 48 hours and weighed to the nearest 0.0001 g. The number of grains in the pollen sample was determined by serial dilutions in water. Weighed pollen was dispersed in 2 ml H_20 and a 20 μl aliquot (*a1*) was diluted to a total volume of 200 μL. The number of grains in a 10 μL aliquot (aliquot2) of this dilution was estimated by counting 20 fields on a 400 field microscope slide at 40x magnification. The calculations used to estimate number of grains per gram were

$$m_{a1} = (m_p)\cdot(0.0005),$$

and

$$\text{Pollen Grains/gm} = ((g/\text{grid})\cdot 400)/(m_{a1}),$$

where m_{a1} = mass of pollen in aliquot 1;
m_p = mass of pollen in original sample;
and g = average number of pollen grains.

The number of pollen grains per strobilus was estimated by multiplying the average mass of pollen per strobilus by the number of pollen grains per gram. Average pollen production per branch segment was estimated for each tree by multiplying average strobilus production per 20 cm branch segment by the average number of pollen grains per strobilus. Population means were the means of all trees in the population for which strobilus production was estimated.

Female Strobilus and Seed Production

Female strobilus production was estimated on approximately half of the study trees in 1992. Four branches were selected from the lower crown of each tree and branch tips were measured 30 cm from the branch apex. In May, June and July, ovules were counted and categorized as 'developing' and 'nondeveloping.' Developing ovules were green and expanded, while nondeveloping ovules were unexpanded and yellow.

In 1993 8 branch tips were selected on each tree by stratified random sampling. Each tree crown was first divided into upper and lower sectors and 4 branches were randomly selected from each sector. Beginning at the basipetal portion of the branch, successive bifurcations were randomly chosen until arriving at a branch apex. Branches were measured 30 cm basipetal from the selected apex and marked with nylon flagging. All branch tips were sampled acropetal to the 30 cm mark. In May, June and July of 1993 the number of ovules (approximately the same as number of strobili, as *T. brevifolia* is primarily uniovulate) was counted and categorized as developing,

nondeveloping or mature, based on external morphology. Developing ovules were green and expanding, while nondeveloping ovules were unexpanded and yellow.

From August until October of 1993 the number of seeds per branch was counted at two-week intervals, and developmental stages were quantified according to the stages defined in Table 2.2. Estimates of cumulative seed production included the total number of mature seeds observed, the total number of empty receptacles (indicative of removal of mature seeds) and the number of healthy, developing seeds present at the final census. Mature seeds and empty receptacles were removed at each census in order to avoid duplication in counting.

Table 2.2 Developmental stages of female strobili, defined for this study.

Stage	Description
0	Scales closed; virtually indistinguishable from vegetative bud.
1	Micropyle visible.
2	Micropyle sealed.
3	Aril visible.
4	Aril covering more than half the length of the seed.
5	Fully formed aril, mature seed (usually brown).

Ovule and seed production in 1994 was estimated in much the same way as in 1993, except trees were visited once per month rather than every 2 weeks. In May of 1994 30 cm was remeasured on each selected branch tip and new branches were selected to compensate for mortality (≈1% of all branches).

In late September of 1994 all branches were clipped at the 1994 flagging and transported to the lab. All growth distal to 1994 bud scars was clipped and separated. All branch material was dried at 65° C for 48 hours and stems, foliage and 1994 growth were weighed separately.

Cosexuality

The frequency of cosexuality (the occurrence of male and female strobili on the same plant, Lloyd 1980) was estimated by surveying 8 30-cm branch sections on each female tree in May and September of 1994 and 8 30-cm branch sections on each male tree in July and August of 1994. The number of male and female strobili was recorded for each branch section.

Sex Ratios, Pollen-ovule Ratios and Reproductive Effort

The genet sex ratio of each population was estimated by counting the number sexually mature male and female *T. brevifolia* trees separated by at least 2 meters. Average floral sex ratios per population were calculated from the ratio of the average male strobilus production/gram of branch to average female strobilus production per gram of branch. Pollen-ovule ratios were calculated as the average number of pollen grains produced per gram of branch divided by the average number of ovules per gram of branch. Average male reproductive effort was indexed for each population by multiplying average production of viable strobili per gram of branch by the site-average strobilus weight. Seed weight per gram of branch was used as an index of average female reproductive effort and was estimated for each population as follows: the average dry weight (65° c, 48 hours) of 10

randomly selected mature female strobili (including arils and receptacles) multiplied by average seed production per gram of branch (on branches bagged to exclude predators). Data from 1994 were used for all calculations.

The above estimates involved several key assumptions beyond those normally made when sampling from a statistical population. For genet sex ratios, stems separated by 2 m or more were assumed to be separate genets. This assumption was probably invalid in some cases because multiple sprouts can arise from the same genet, and connections between the sprouts can degenerate over time. Assumptions in the calculation of floral sex ratios, pollen-ovule ratios, and reproductive effort were as follows: *i.* estimates of strobilus production based on branch subsamples were consistently proportional to the productivity of entire trees; *ii.* the size of the primary branches was unrelated to the intensity of strobilus production; *iii.* there were no systematic differences in the size and morphology of male and female canopies; and *iv.* 20 cm branch segments contained the same proportion of nonreproductive biomass as 30 cm branch sections. If any of these assumptions were violated then the estimates of strobilus production, floral sex ratios, pollen-ovule ratios and reproductive effort would be biased. Therefore, these data should be interpreted with caution.

Seed Mass and Viability

In order to evaluate differences in seed size among sites, 2-5 trees were randomly selected from each canopy grouping per site, excluding the VAL CAN population (in

which no seed production was observed). Seed was collected throughout the summer and fall of 1993. Arils were removed using a blender with blades shielded by rubber tubing, and dry mass was determined for 10 seeds from each tree.

Seed viability was determined by three different methods: x-rays, tetrazolium staining of the embryo, and cutting the seed open to examine the embryo and megagametophyte. The different methods yielded contrasting results within seed lots and even with the same seeds. The tetrazolium test was the most conservative and the x-ray test the most liberal. The cut test was chosen because it was the simplest and least ambiguous, and because it has been shown to correlate well with seed and embryo germination (Pilz unpublished; Vance *et al.* in preparation). The test was performed on 10 randomly selected seeds from each of the trees used in determination of seed mass. Viability was assumed if the megagametophyte completely filled the seed and the embryo was opaque and fully formed.

Phenology of Pollen Shedding

In order to estimate the length of the pollination season and assess variability in pollen shedding, 9-13 trees per site were selected for intensive study. 5 branches were randomly selected from throughout the crown of each tree and 50 to 100 cm branch segments were marked with nylon flagging. Beginning before the onset of pollen shedding, trees were visited every 3 to 5 days to record percent strobili that had shed pollen. Because it was extremely difficult to determine if individual strobili were actively shedding pollen (particularly when strobili were wet),

actively shedding strobili were not differentiated from those that had completed pollen shedding. Consequently, estimates of pollen shedding ranged from 0 to 100 percent shed. Pollen shedding was considered to be effectively complete when more than 95 percent of the viable strobili had shed pollen, and agitated branches did not produce a detectable pollen cloud.

Phenology of Female Anthesis

Female anthesis was estimated on 5 trees at HC1 and 5 trees at VAL in 1994. Anthesis was defined as the emergence of the ovule from beneath the bud scales. The number of visible ovules on 10 branches was counted at 3-7 day intervals during the time of peak pollen shedding. The presence of pollination drops was also noted.

Phenology of Seed Maturation

Assessment of seed maturation rates was complicated by removal of mature and immature seed by predators. If removal rates varied spatially and temporally, then simply counting ripe seeds every two weeks would be misleading. This problem was accounted for by counting both mature (Stage 5) seeds and seed receptacles, which are left behind after seeds are removed. This method is also somewhat biased because receptacles sometimes remain when immature (Stage 4) seeds are removed, so maturation rates may be overestimated.

Variation in rates of development was also assessed from the range of developmental stages present on bagged branches at the final censuses of 1993 and 1994. It is not known how bags affected developmental rates, but a

comparison with unbagged branches is not valid because of the confounding effects of seed removal.

Overstory Canopy

Overstory canopy openness above and to the south of each study tree was estimated using the model LAI-2000 plant canopy analyzer (LI-COR, Lincoln, NE, USA). This instrument derives estimates of canopy cover from measurements of diffuse radiation. A refernce sensor placed in a clearing automatically logged light readings every 30 s while readings were taken above each *T. brevifolia* tree with a measurement sensor. Both sensors were levelled and pointed in exactly the same direction. Because of the difficulty in finding sufficiently large open areas near the *T. brevifolia* populations, a 270° lens cap was used on both sensors. Measurements were taken in a southwesterly direction between 7 am and 11 am and in a southeasterly direction between 4 pm and 8 pm, because direct sunlight confounds readings. All measurements were taken under relatively uniform sky conditions, usually on cloudless days. Most measurements were taken during July and August of 1993.

The value used in this study was Diffuse Noninterceptance (DIFN), an estimate of the amount of sky visible from beneath the canopy. DIFN ranges from 0 (completely closed canopy) to 1 (no canopy) (Welles and Norman 1991). The average of the southwest and southeast DIFN readings is an estimate of the openness of the canopy directly above and surrounding the southern aspect of each *T. brevifolia* tree. The southern aspect was measured on the assumption that this was the location of the most important

canopy gaps at this latitude (i.e. those that resulted in the most significant increases in understory light levels).

Analysis

Two-by-two frequency tables were used to determine the significance of deviations of sex ratios from unity.

Linear regression and ANOVA were performed using SAS statistical software (SAS Institute 1987). Data were transformed prior to performing analyses to correct for heteroscedasticity. Fisher's Protected LSD was used for multiple range comparisons. This is a somewhat liberal method of comparison, so care should be taken in interpretation of differences (SAS Institute 1987).

In order to examine the relative importance of year-to-year variation in relation to variation among populations, among trees and within trees, variance components were calculated for male and female strobilus production for 1993 and 1994 (VARCOMP procedure, SAS Institute 1987).

In order to assess the significance of interannual differences in strobilus and seed production, pairwise comparisons of individual tree means were made using the Wilcoxon Signed-Rank Test (Mosteller and Rourke 1973). ANOVA was not used for interannual comparisons because the errors of the estimates were not independent between years. Interannual correspondence of ranks of average tree fecundity was assessed with Kendall's coefficient of correspondence (τ_b) (Kendall 1970).

RESULTS

Cosexuality

Of the 115 trees specifically checked for the occurrence of cosexuality, 98 were strictly male or female and 17 predominantly male (male-function) trees (15%) produced some female strobili. This represented 29% of all male-function trees checked (Table 2.3). No male strobili were found on predominantly female *T. brevifolia* trees.

The occurrence of cosexuality varied among sites, and may have been related to elevation. Only 1 of 14 male-function trees (7%) examined at the Willamette Valley site (VAL) exhibited cosexuality, while 14 of 44 male-function trees (33%) from the Cascade sites (HC1, HC2, LC) were cosexual (Table 2.3). Beyond this possible relationship, there was no obvious evidence of microenvironmental control of sex expression. Trees growing in close proximity under similar overstory canopy did not necessarily have the same sex expression.

The production of female strobili by cosexual trees occurred on multiple branches on most of the trees for which the phenomenon was observed. Male and female strobili occurred together on the same branch segments, and there was no pronounced sectorization. The number of ovules produced by cosexual trees was low relative to production of male strobili by cosexual trees and production of ovules by single-sex female trees (Table 2.3). There was a trend for cosexual trees to produce fewer male strobili than unisexual male trees, but the difference was not significant (ANOVA

with population as blocking factor, main effects F=0.72, p=0.23, n=60).

Population Sex Ratios and Pollen-ovule Ratios

Genet sex ratios were mostly close to 1:1, with male bias being more common than female bias (Table 2.4). However, the only population in which the sex ratio was significantly different from unity was the OPN population at site HC2 (χ^2=19.9; p<0.001). Many of the male genets on this plot consisted of clumps of sprouts resulting from layering of downed *T. brevifolia* trees. However, even when large trees were considered exclusively, males still outnumbered females at this site (personal observation).

Table 2.3 Occurrence of cosexuality in *T. brevifolia* populations. Freq. Cosex.- Frequency of cosexual males/number of male-function trees checked; ♂ Strobili ♂- average number of male strobili/20 cm branch segment on unisexual males; ♂ Strobili ♂♀- average number of male strobili/ 20 cm branch segment on cosexual trees; Ovules ♂♀- average number of ovules/30 cm branch segment on cosexual trees. Ovules ♀-average number of ovules per 30 cm branch segment on unisexual females.

Site	Pop.	Freq. Cosex.	♂Strobili ♂	♂Strobili ♂♀	Ovules ♂♀	Ovules ♀
HC1	OPN	2/7	81.45	55.75	1.125	19.93
	CAN	5/7	33.88	15.15	1.000	15.93
HC2	OPN	5/9	73.96	49.25	0.275	15.04
	CAN	0/7	12.63	NA	NA	NA
LC	OPN	1/7	42.11	19.75	0.125	12.64
	CAN	3/7	8.78	10.50	0.420	2.87
VAL	OPN	1/7	80.75	29.50	0.125	19.89
	CAN	0/7	7.95	NA	NA	NA

NA- Not Applicable.

The floral sex ratio index was consistently male-biased for all populations, with males producing from 5 to 12 times more strobili than females per gram of branch sample (Table 2.4). Males also produced more than a million times more gametes than females at all sites, as indicated by pollen-ovule ratios. However the index of female reproductive effort (RE, estimated from mass of reproductive structures) was approximately 5 times greater than male RE at the Cascade sites. Female RE was also higher than male RE for the VAL OPN plot population, but the ratio approached 1 in this population. The calculation could not be made for the VAL CAN plot population because no mature seed was observed.

Table 2.4 Sex ratios per population. Calculations are described in methods. All ratios were calculated as male:female. Male-function cosexual trees were counted as males. P-O- Pollen-Ovule; RE- Reproductive Effort, based on mass of male strobili and mature female strobili. Floral, P-O, and RE ratios were normalized by branch weight, and population means were calculated from individual tree means.

Site	Pop.	N	Genet Sex Ratio	Floral Sex Ratio	P-O Ratio ($x10^6$)	RE Ratio
HC1	OPN	23	1.09	11.76	5.4	0.17
	CAN	63	1.33	5.35	2.5	0.25
HC2	OPN	187	1.97*	10.16	6.1	0.18
	CAN	22	1.20	7.46	5.6	0.23
LC	OPN	52	1.36	7.96	4.7	0.25
	CAN	33	0.83	8.83	6.1	0.13
VAL	OPN	40	0.82	5.55	2.9	0.90
	CAN	54	1.07	12.19	6.3	—[1]

* Significantly different from 1 ($\chi^2=19.6$; $p<0.001$).
[1] No mature seed observed in this population.

Seed Mass and Viability

Seed mass generally decreased with elevation (Table 2.5; Figure 2.1). Seeds collected from trees in the Willamette valley had significantly greater mass than those from the two higher elevation Cascade sites. Seeds from the lower elevation Cascade site had intermediate mass (Table 2.5). Differences in seed mass among plots within sites were not significant (data not shown). Mean percent viability by site was no lower than 80%, as determined by cut tests. Viability was significantly lower at the VAL site than for the HC2 and LC populations (Table 2.5).

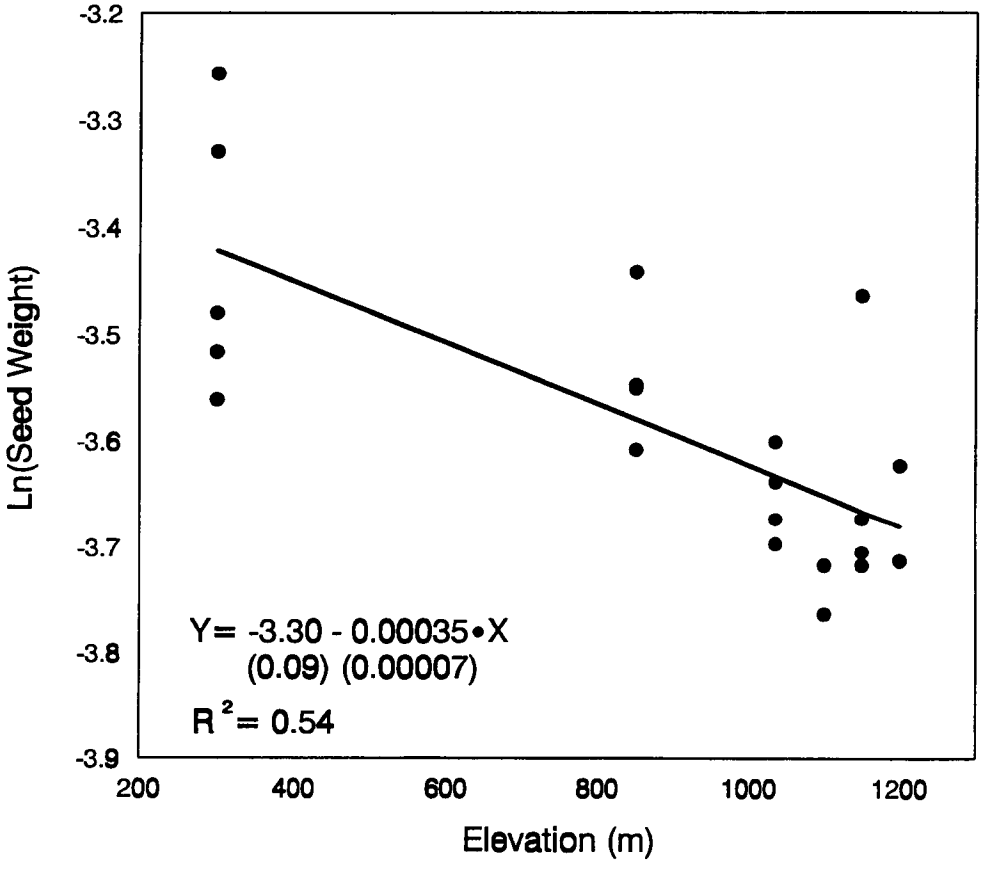

Figure 2.1 Regression of seed mass on population elevation. Points represent average seed dry mass for individual trees. Equation is best fit from least squares regression, with standard errors for parameter estimates in parentheses. Mass was natural log transformed to correct for heteroscedasticity.

Table 2.5 Seed mass and viability. Values in parentheses are standard errors. Letters indicate homogeneous groups as determined by Fisher's protected LSD (p=.05, SAS Institute 1987). Analyses were performed on natural log (seed mass) and arc sine square root (viability) transformed data. Back-transformed values are presented.[1]

Site	N	Seed Mass (g)	Viability (%)
HC1	4	0.025(0.0014)^{ab}	85(10)^{ac}
HC2	8	0.026(0.0023)^{ab}	98(5)^{ab}
LC	4	0.029(0.0021)^{bc}	100(0)^{b}
VAL	5	0.033(0.0043)^{c}	80(23)^{c}

[1] Standard errors are approximate values from back-transformations.

Phenology of Pollen Shedding

Pollen shedding began in early March and extended into late April in 1994. The timing of pollen shed by males was related to elevation and overstory canopy openness (Figure 2.2). The Willamette valley populations differed significantly from the Cascade populations in date of onset of pollen shedding (Table 2.6). The first two populations to shed in the Cascades were LC OPN and HC1 OPN. The onset of shedding for these populations was significantly different from that of the HC2 CAN population, which was the last population to begin shedding pollen (Table 2.6; Figure 2.2). There were no significant differences in onset of pollen shedding among the Cascade CAN populations. Also, although the difference was only significant at site LC, OPN populations began shedding pollen at an earlier date than CAN populations at all 4 sites (Table 2.6). This was true even at sites HC2 and VAL, where OPN populations occurred at

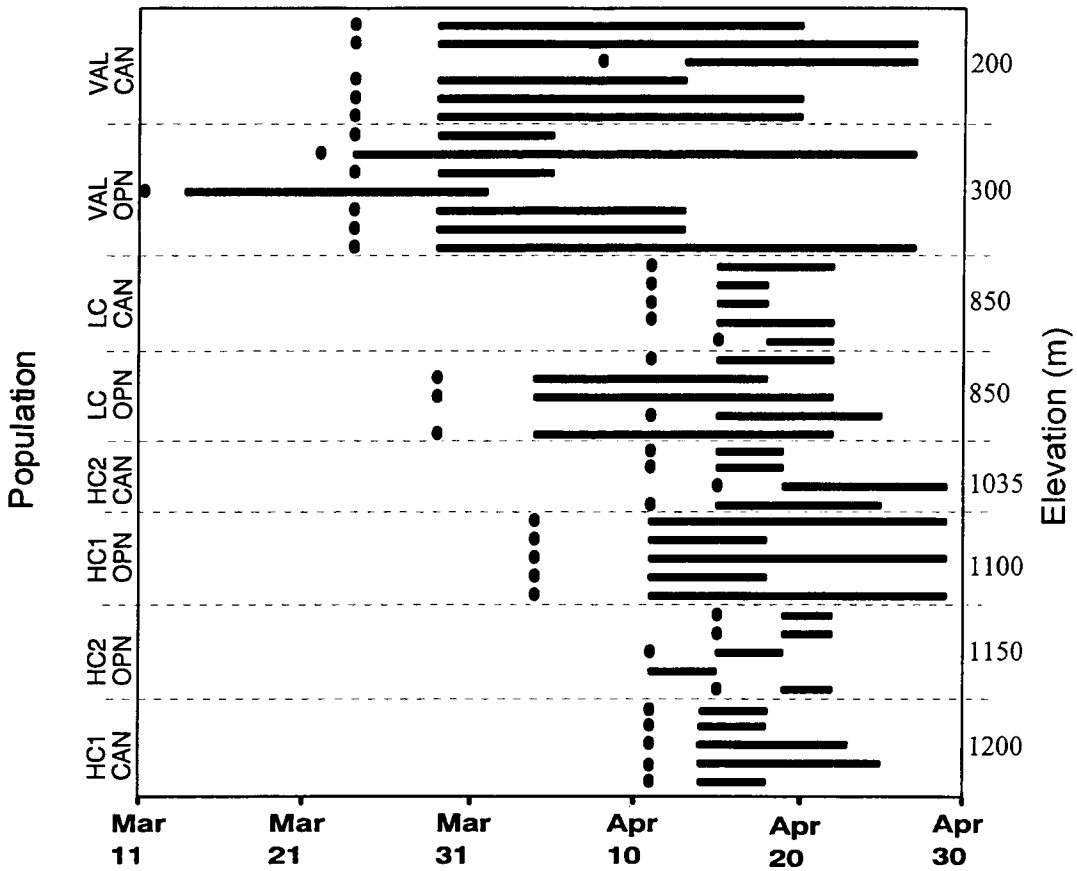

Figure 2.2 Phenology of pollen shedding in 1994. Ovals represent last date on which trees were checked and no shedding was observed. Horizontal bars represent duration of observed pollen shedding.

100 m lower elevation than CAN populations (Table 2.6, Figure 2.2).

Onset of pollen shedding also varied within trees: the highest branch on each tree shed earlier than the lowest branch (Wilcoxon Signed-Rank Test, W=11.69, p=0.0001, n=42).

The duration of pollen shedding varied among populations as well, ranging from about 3 to 20 days on average (Table 2.6, Figure 2.2). The duration of active pollen shedding was greater on average in the VAL populations and in Cascade OPN populations than in Cascade

CAN populations. One exception was the HC2 OPN population, which was not significantly different in duration from the Cascade CAN populations (Table 2.6).

Table 2.6 Comparisons of male phenology among populations. Standard errors are in parentheses. Letters indicate homogeneous groups as determined by Fisher's protected LSD (p=0.05, SAS Institute 1987). Mean duration was natural log transformed.[1]

Site	Population	N	Mean Onset of Shedding (Julian Day)	Mean Duration of Shedding (Days)
HC1	OPN	5	101(0)^{ac}	13.6(1.3)^{a}
	CAN	5	104(0)^{ab}	6.4(1.3)^{b}
HC2	OPN	5	105(1.3)^{ab}	6.4(1.3)^{b}
	CAN	4	108(1.0)^{b}	3.3(1.1)^{b}
LC	OPN	5	98(2.7)^{c}	13.4(1.2)^{a}
	CAN	5	106(0.6)^{ab}	4.8(1.2)^{b}
VAL	OPN	7	85(2.1)^{d}	17.9(1.3)^{a}
	CAN	6	91(2.5)^{d}	20.7(1.1)^{a}

[1] Standard errors are approximated by back-transformed values.

Phenology of Female Anthesis

The phenology of anthesis of female strobili varied among the 5 trees examined at each site (Figures 2.3, 2.4). On March 17 in the VAL OPN population, one of the trees had nearly all ovules visible, while another was intermediate and the third tree had anthesed a small proportion of the ovules that would eventually appear (Figure 2.4A). Female anthesis began well before most males began shedding pollen in the VAL OPN population. Also, some pollination drops

were observed after pollen shedding in the population had effectively ceased (Figure 2.4A). This may indicate that female receptivity was not entirely synchronous with pollen shedding of the males examined. Similar patterns were observed in the VAL CAN and HC1 OPN populations. Although there was less variation in female anthesis than in the VAL OPN population, females in these populations also appeared to begin anthesis before the onset of pollen shedding and continued to produce pollination drops after shedding was effectively complete (Figures 2.3, 2.4B).

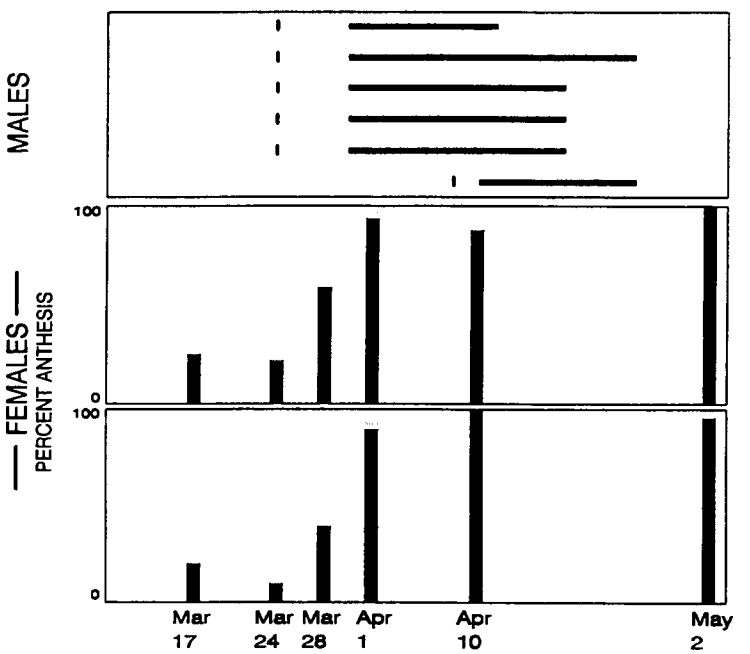

Figure 2.3 Pollination phenology at the HC1 site, 1994. The male graphs are the same as in Figure 2.2. Each female graph represents anthesis and receptivity of ovules on a single tree. Black bars represent the number of ovules observed on each date, relative to total ovules visible in May. Gray shading represents visible ovules with pollination drops present. Decreases in the number of visible ovules were probably due to errors in counting.

Phenology of Seed Development

Mature seeds were first observed in July at all sites in all three years of the study. There was variation in the rates of mature seed production among trees within populations, among populations within sites and among sites (Figure 2.5). A higher proportion of seeds matured during the early part of the season in the VAL and LC populations than in the Higher Cascade populations, as indicated by earlier dates of 50% seed maturation (Figure 2.5). Also, the HC1 CAN population reached 50% mature seed production in the third week of August while the HC1 OPN population did not reach 50% until the second week of September.

A proportion of the apparently healthy seeds produced by each tree never reached maturity. A range of developmental stages were present on all trees at the final censuses in early October of 1993 and late September of 1994 (Figure 2.6). Although some development continued into November and December, especially at the VAL site, many seeds failed to complete development (personal observation).

There were no significant differences in the proportion of mature (Stage 5) seeds between populations within sites in 1993 and 1994, but some lower elevation populations had smaller proportions of mature seeds than higher elevation populations (Figure 2.6, Table 2.7). A higher proportion of mature seed was present on bagged branches in the HC2 OPN population than in the LC CAN population in 1993. In 1994 there were no significant differences among populations in mature seed percentage, but the trends were similar to those

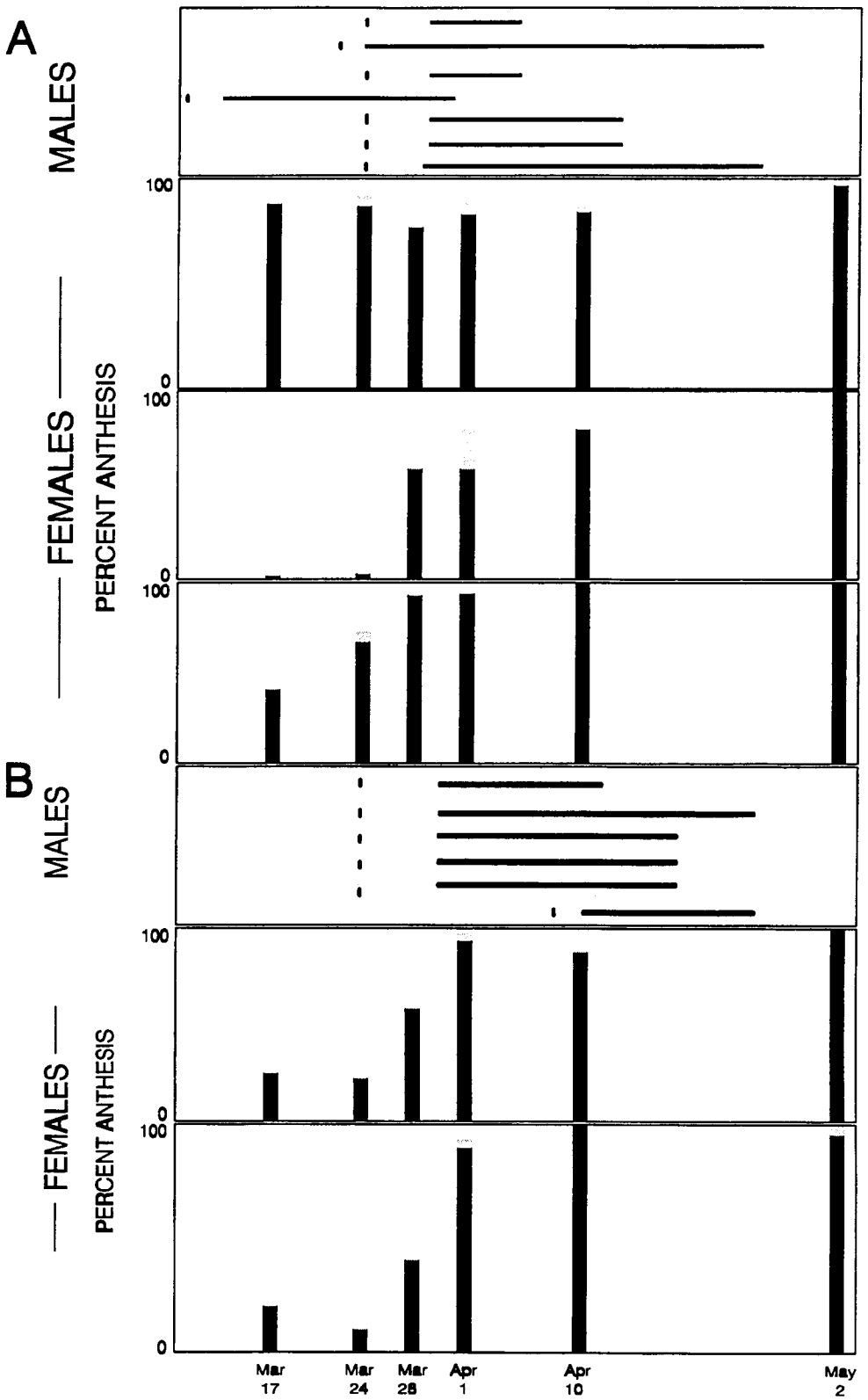

Figure 2.4 Pollination phenology at the VAL site, 1994. **A.** VAL OPN population. **B.** VAL CAN population. See Figure 2.3 for explanation.

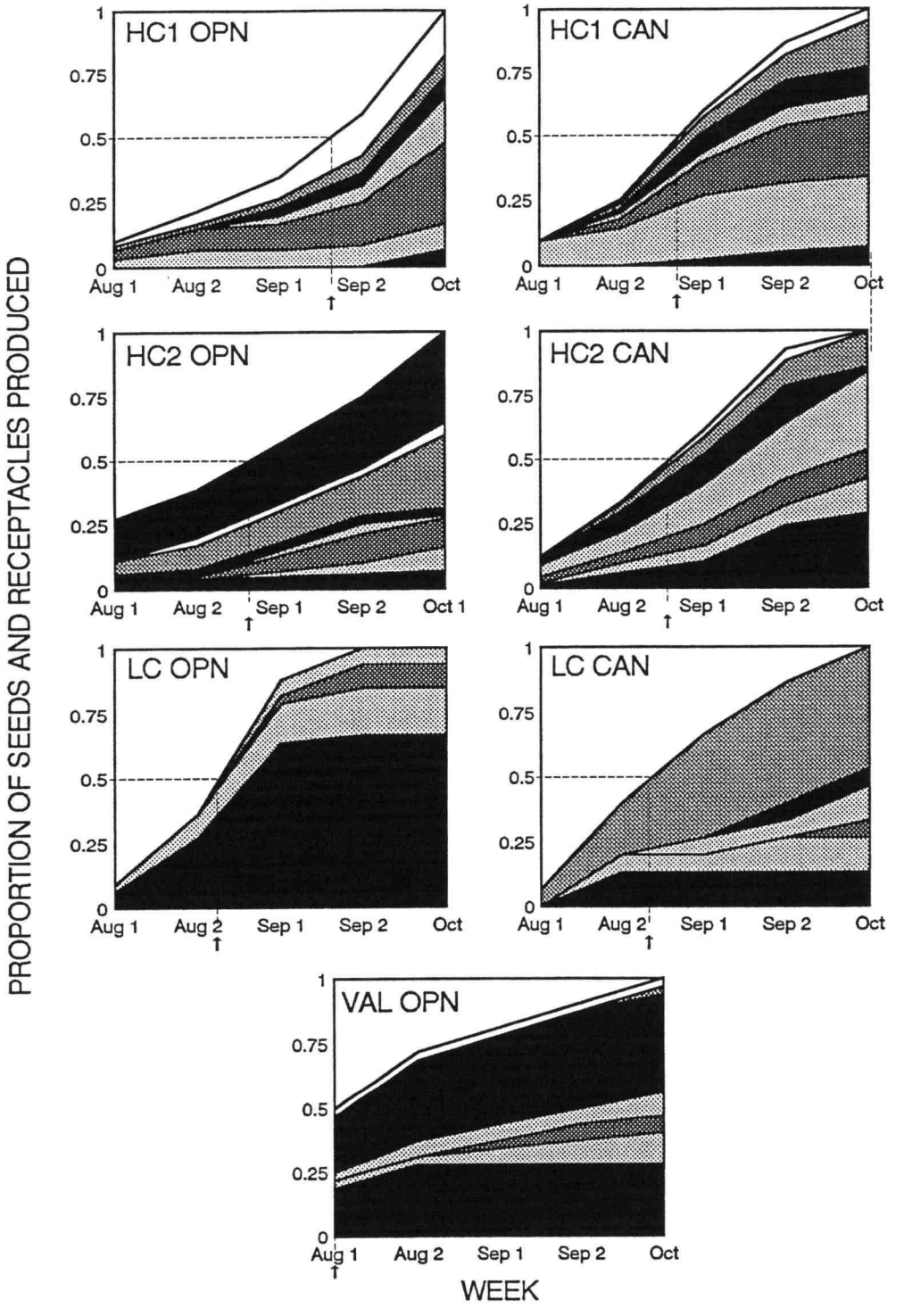

Figure 2.5 Phenology of mature seed production in 1993. Different shading patterns represent the number of mature seeds and empty receptacles observed on each tree over time, as a proportion of the total number of mature seed and receptacles observed in the population up to October.

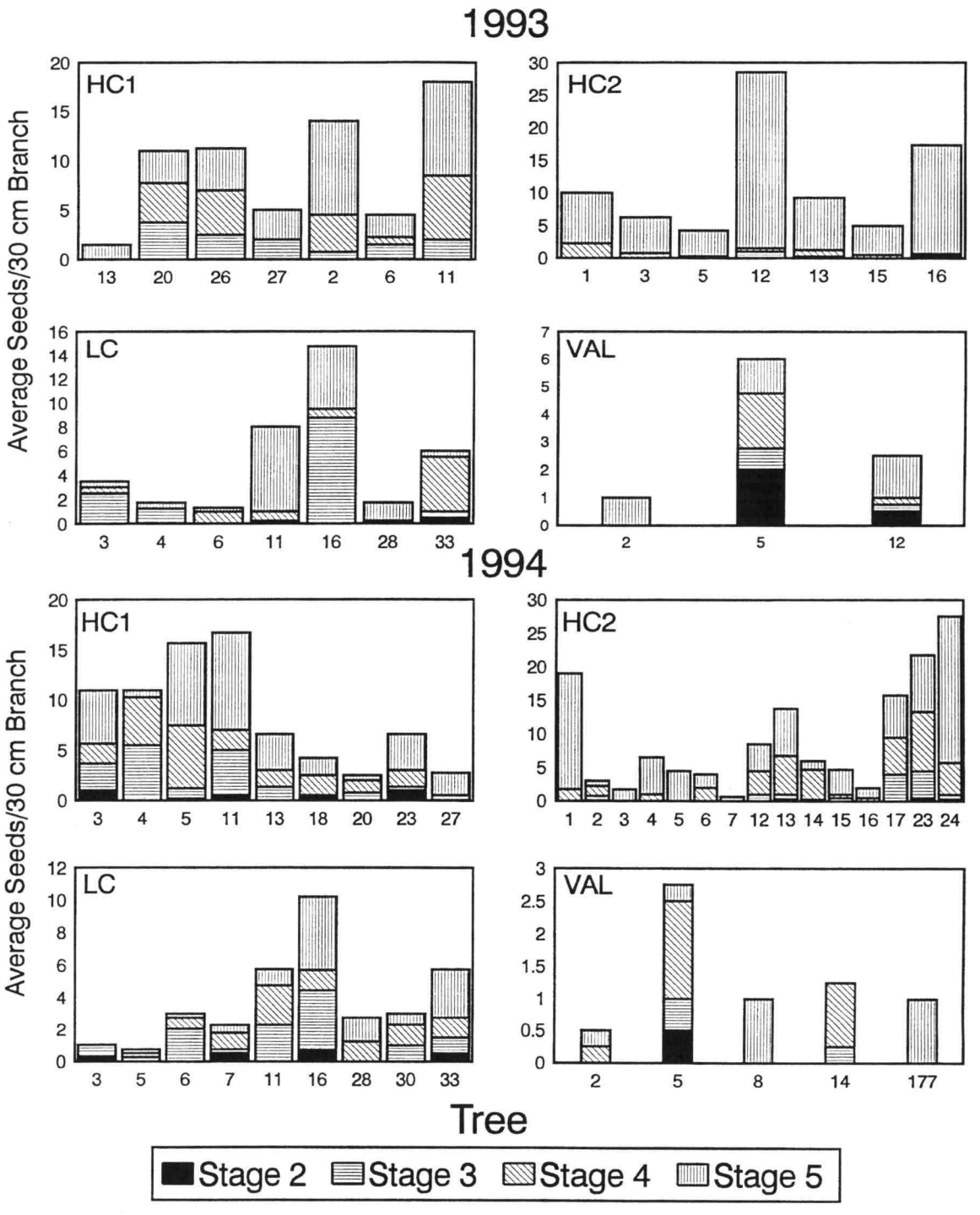

Figure 2.6 Developmental stages present at final censuses in 1993 and 1994. Each bar represents tree averages for branches bagged to exclude predators. Numbers below bars identify trees. Developmental stages are as described in Table 2.2.

in 1993 (Table 2.7). The trends were for more advanced development in the HC2 populations than in the HC1, LC and VAL populations in both years. This effect might have been caused by a later census date (7-13 days after the other sites) for HC2 in 1993. However, the trends were present in 1994 as well, when all populations were censused within a three day period.

Variation in Strobilus Production

Analysis of variance components indicated that the largest source of variation was within trees, followed by among trees and then among plots. Among-year variation was minor compared to variation from other sources (Table 2.8).

Year-to-year variation was further analyzed by comparison of paired means from individual trees (Table 2.9), which eliminates the effect of variation among trees. With all sites combined, male strobilus production was not significantly different among years. Productivity at the VAL site was significantly greater in 1994 than 1993, and 1992 productivity at HC2 was less than 1993 and 1994. The other seven population comparisons revealed no significant differences among years in male productivity (Table 2.9).

Female strobilus production showed more variability than male strobilus production among years. For all sites combined, 1994 production was significantly less than 1993 production, and nearly significantly less than 1992 production ($p=0.06$). 1993 female strobilus production was significantly greater than 1994 production at all 3 Cascade sites. In addition, 1993 productivity at HC2 was greater than 1992, and 1992 was greater than 1994 at HC1. In total,

Table 2.7 Proportion of seeds present on bagged branches that were mature as of the final census in 1993 and 1994. Proportions are site averages of tree averages for branches bagged to exclude predators. Day is Julian day. Values in parentheses are standard errors. Letters indicate homogenous groups within years, as determined by Fisher's protected LSD (p=0.05).

		1993			1994		
Site	Pop.	N	Day	Mature Seed(%)	N	Day	Mature Seed(%)
HC1	OPN	3	270	56.9(5.5)^{abc}	4	264	42.4(8.2)^{a}
	CAN	4	270	56.9(15.8)^{abc}	4	264	41.3(11.8)^{a}
HC2	OPN	4	280	93.1(2.2)^{c}	7	264	54.2(8.8)^{a}
	CAN	3	280	86.5(4.9)^{bc}	7	264	71.6(11.1)^{a}
LC	OPN	4	273	54.3(19.5)^{ab}	7	263	45.1(10.7)^{a}
	CAN	3	273	16.7(4.8)^{a}	4	263	30.9(18.3)^{a}
VAL	OPN	3	267	60.0(22.9)^{abc}	5	262	51.8(21.3)^{a}

Table 2.8 Components of variation in male and female strobilus production for 1993 and 1994.

	Variance Components	
Source	Females	Males
Year	0.76	0
Plot	25.94	106.91
Tree	128.98	847.42
Branch	152.09	1219.69

5 out of 12 population comparisons were significant for female strobilus production (Table 2.9).

In contrast to female strobilus production, seed production did not vary between years with all sites combined. Also, there was poor correspondence between strobilus and seed production for interannual differences

Table 2.9 Comparison of male and female strobilus production among years. W is Wilcoxon Signed Rank score, and associated p-value (Mosteller and Rourke 1973). τ_b is Kendall's Coefficient of correspondence for comparing rankings of tree productivy between years (Kendall 1970). Comparisons with 1992 data were done for strobilus production normalized by branch weight. 1993 strobilus production was normalized by average 1994 branch weight for these comparisons.

	1994-1993				1994-1992				1993-1992			
	N[1]	W	p	τ_b	N[1]	W	p	τ_b	N[1]	W	p	τ_b
Site	Male Strobilus Production											
HC1	14	-1.64	0.49	0.63	6	-0.16	0.93	ns	6	-0.16	0.93	ns
HC2	16	-1.38	0.59	0.77	6	3.17	0.03	ns	6	3.50	<0.01	ns
LC	14	2.71	0.25	0.81	ND	ND	ND	ND	ND	ND	ND	ND
VAL	21	2.77	0.01	0.68	8	2.00	0.29	0.64	8	0.50	0.80	0.57
All Sites	65	4.51	0.34	0.72	20	4.30	0.11	0.64	20	3.30	0.23	0.69
	Female Strobilus Production											
HC1	14	-6.86	<0.01	0.54	6	-3.17	0.03	ns	6	1.17	0.51	ns
HC2	15	-4.53	0.05	0.64	7	-0.08	0.97	ns	7	4.69	0.02	ns
LC	14	-4.57	0.04	0.68	13	-0.86	0.65	ns	13	2.71	0.11	ns
VAL	14	-2.14	0.37	0.72	5	-1.8	0.27	ns	5	-1.40	0.40	ns
All Sites	57	-17.00	<0.01	0.71	31	-6.03	0.06	0.51	31	4.29	0.20	0.57
	Seed Production											
HC1	14	-3.35	0.15	ns	6	2.50	0.11	ns	6	2.50	0.12	ns
HC2	15	0.40	0.87	ns	7	4.23	0.05	ns	7	4.86	0.03	ns
LC	14	5.86	<0.01	ns	13	-0.71	0.70	ns	13	-3.42	0.03	ns
VAL	14	-0.79	0.72	0.61	5	-2.60	0.07	ns	5	0.20	0.91	0.89
All Sites	57	1.44	0.75	0.33	31	3.16	0.12	ns	31	5.25	0.12	ns

[1]Number of trees used in comparison. Trees from comparisons involving 1992 data are a subset of those used in 1994-1993 comparison. ND- No data; ns- τ_b not significant at $p \leq 0.05$.

for individual sites (Table 2.9). For 5 of 7 comparisons in which there were significant differences in strobilus production, there were no significant differences in seed production. Also, for 3 of 4 comparisons in which there were significant differences in seed production, there were no significant differences in strobilus production. In one of the two cases where the same comparison was significant for both strobilus and seed production (1994-1993, LC), the direction of the difference in strobilus production was reversed for seed production (Table 2.9).

As indicated by Kendall's coefficient of correspondence (τ_b, Kendall 1970), the ranking of tree productivity remained fairly constant between 1993 and 1994 for male and female strobilus production. However, there was relatively poor correspondence between years for seed production (Table 2.9). Comparisons with 1992 productivity showed poor correspondence for all three values, though rankings for all sites combined showed significant correspondence for male and female strobilus production in 1994-1993 and 1993-1992 comparisons. There were no significant negative correspondences in productivity, meaning productive years were not typically followed by unproductive years for individual trees, or vice versa, over the three years of this study.

DISCUSSION

Breeding System

T. brevifolia is not strictly dioecious, as was previously reported (e.g. Bolsinger and Jaramillo 1990; Hitchcock and Cronquist 1973; Sargent 1933). The occurrence

of cosexuality appears to be widespread enough to warrant changing the breeding system designation of *T. brevifolia* from 'dioecious' to 'subdioecious' (Darwin 1877) or functionally dioecious with 'leakage.' The rate of occurrence of cosexual trees observed in this study is comparable to that observed in the USDA Bureau of Land Management (BLM) Pacific yew inventory (Anonymous 1992b). Of 1067 trees for which sex was determined in the BLM inventory, 107 (10%) displayed cosexuality (unpublished data). By comparison, 15% percent of the 115 trees examined in the present study displayed cosexuality. The difference in percentage could be due to sampling error, or to differences in the number of branches examined on each tree.

All of the cosexual trees examined in this study had predominantly male sex expression, and the number of ovules produced by cosexual trees was negligible in comparison to male strobili on cosexual trees or ovules on female trees. In addition, cosexual trees produced fewer strobili than unisexual trees in 5 out of 6 populations. Although the difference was not significant, this pattern suggests that there may be a tradeoff between male and female strobilus production for cosexual trees. That is, male and female strobilus production might be constrained by the same pool of resources, and production of female strobili comes at the expense of male strobilus production. Alternatively, this pattern might indicate that cosexuality is favored under conditions unfavorable for male strobilus production.

The pattern of leakage of female sterility but not of male sterility has also been observed for other putatively dioecious *Taxus* species, including *T. cuspidata*, *T. baccata*,

and various hybrid *Taxus* cultivars (Chadwick and Keen 1976; Pridnya 1984). The same pattern has also been observed in other dioecious plants in the Myristicaceae (Armstrong and Irvine 1989), Caryophyllaceae (Sakai and Weller 1991) and Monimiaceae (Feil 1992), for species that were all woody understory plants with fleshy fruits. Similarly, gynodioecy, the presence of female plants and hermaphrodites, is a much more common breeding system than androdicioecy, the presence of males and hermaphrodites (Willson 1983).

It has been suggested that leakage of male sterility is sometimes not observed due to the inconspicuous nature of the male reproductive structures relative to seeds and fruits (Feil 1992; Keen and Chadwick 1954). This is probably not the case in the present study, because female trees were examined numerous times throughout the season, including during pollen shedding when male strobili were quite conspicuous. If the survey was biased, it was in favor of detecting leakage of male sterility rather than of female sterility.

It is significant in the light of theories about the evolution of dioecy that *T. brevifolia* cosexual trees have predominantly male sex expression. Dioecy is likely to have evolved from monoecy in gymnosperms, as the majority of gymnosperms are monoecious and this appears to be the ancestral condition (Givnish 1980). Dioecy has been shown to correlate strongly with woody habit, wind pollination, and fleshy fruits (Bawa 1980; Givnish 1980; but see Muenchow 1987), all of which are characteristic of *Taxus*. Givnish (1980) and Bawa (1980) hypothesized that this positive

correlation provides clues to the mode of evolution of dioecy in gymnosperms. Wind pollination yields diminishing returns for increased investment in male function due to presumed restricted movement of pollen. On the other hand, if seed dispersers are attracted by large fruit crops, investment in fleshy fruits would bring strong gains in fitness. In a situation where there is a tradeoff between investment in male and female function, male sterile mutants would be strongly favored in a population of cosexual plants. As unisexual female plants become more common and experienced disproportionate success in dispersal of fruit crops, hermaphroditic female function would be selected against because it would represent a drain on scarce resources for diminishing returns. This scenario suggests that male function would be strongly selected against in hermaphrodites first, and subsequently female function would be selected against in hermaphrodites.

The observation that male sterility is a more consistent trait than female sterility in *T. brevifolia* may support the above scenario. If male function were strongly selected against and lost first, followed by subsequent selection against female function, one might expect to see more leakage in female sterility. Dioecy is thought to have evolved from monoecy by gradual shifts in floral sex ratios within plants (Charlesworth and Charlesworth 1978). These shifts might have occurred through changes in the frequency of genes controlling the conversion of flowers from one sex to another (Ross 1982). One possible scenario is that all *T. brevifolia* generative buds are destined to develop into male strobili unless a female inducing factor is present.

If the gene or genes controlling the hypothetical inducing factor were strongly selected for and became constitutively expressed in some individuals, unisexual females would result. Unisexual males would follow as inhibitors of the female inducing factor became more common, or mutations in the female inducing factor gene(s) became fixed. Although evidence for the existence of such a system of sex determination in *Taxus* is lacking, observations that small individuals of *T. canadensis* tend to be male (Allison 1991) and that some *Taxus* cultivars exhibit sex reversal from male to female (Chadwick and Keen 1976), are consistent with the model outlined above. Of course, several alternative scenarios are also plausible and consistent with the data. More study is required to determine the mode of sex expression in *Taxus*, and whether or not the occurrence of cosexuality has environmental correlates that can shed light on some of the factors favoring the evolution of dioecy.

Genet Sex Ratios

Population HC2 showed a significantly male-biased sex ratio. This population consisted of a large number of small genets, many of which resulted from vegetative regeneration from fallen trees. It is possible that some of these genets actually represented multiple ramets of the same genet, but care was taken to count stems that were physically connected or closer than 2 m in proximity as a single genet. Also, there is no reason to expect that errors in estimates of numbers of genets would be greater for males than for females. Redmond (1984) also found male-biased sex-ratios in several populations of *T. floridana*. One possible

explanation for male bias in population HC2 is that conditions were such at this site that smaller and/or younger individuals tended to be male, and that some genets would change sex as they grew larger, as observed for other *Taxus* species (Chadwick and Keen 1976). Biased sex ratios could also result from differential mortality of the sexes due to dimorphic resource requirements (Freeman *et al.* 1976, 1980; Bierzychudek and Eckhart 1988; Sakai and Weller 1991; Vitale and Freeman 1986; Werren and Charnov 1978). Reproductive effort (based on dry mass) in *T. brevifolia* is considerably higher for females than for males, despite male-biased floral sex ratios. Under stressful circumstances female mortality might exceed male mortality because seed production is more resource-intensive. The former hypothesis could be evaluated by following sex expression of individual genets over time. The latter hypothesis could be evaluated by comparing the microsites occupied by male, female and cosexual trees.

Despite the suggestiveness of the pattern, strong conclusions should not be drawn about male-biased sex ratios in *T. brevifolia* populations. Significant male bias was only observed in a single population, and this may have been anomalous. Also, strong female bias has been observed in some *T. brevifolia* populations (C. Bolsinger, personal communication). Finally, male-biased sex ratios do not seem to be a general phenomenon among *T. brevifolia* populations. In the BLM inventory, a total of 421 male, 539 female and 107 cosexual genets were identified. If one combines the cosexual and male trees, as was done in the present study, the sex ratio approaches unity. Within individual

populations in the BLM survey, female bias was more common than male bias, though this could be a function of the inconspicuousness of the male strobilus to the untrained eye. Sex was determined on a small fraction (less than 1%) of all trees examined in the BLM inventory, and it is quite possible that there was a bias toward recognition of female strobili.

Floral Sex Ratios and Reproductive Effort

Floral sex ratios were consistently male-biased across all populations because numbers of male strobili per branch were several times greater than female strobili on average. In contrast, ratios of reproductive effort were consistently female-biased. This was despite the failure to account for the mass of aborted ovules in measures of female reproductive effort. Male-biased floral sex ratios and female-biased RE ratios are remarkably common across different breeding, pollination and dispersal systems (Sutherland 1986). Such patterns may reflect differential selective pressures on the sexes (*i.e.* sexual selection, Willson 1979).

Pollen-Ovule Ratios

Pollen-ovule (P-O) ratios are strongly correlated with the breeding systems of plant species (Cruden 1977). If P-O ratios are subject to natural selection, they should be inversely related to the efficiency of pollination. Cruden (1977) showed that for angiosperms, P-O ratios were lowest for cleistogamous species, intermediate for autogamous species and highest for xenogamous species. Also, Cruden (1977) showed that P-O ratios correlated well with the

successional stage a species was likely to inhabit, being lowest for pioneer species and highest for species of late successional stages. As a (mostly) obligately xenogamous species typical of late successional stages, one would expect P-O ratios of *T. brevifolia* to be high. In fact, the ratios estimated in this study were in excess of a million, among the highest reported in the literature. For example, P-O ratios of *Lindera benzoin* were mostly less than 100,000 (Niesenbaum 1992), *Siparuna* species ranged from 1000 to 200,000 (Feil 1992), *Solanum appendiculatum* was about 10,000 (Mione and Anderson 1992) and *Myristica insipida* was between 16000 and 19000 (Armstrong and Irvine 1989). The high P-O ratios of *T. brevifolia* may be associated with the selective pressures imposed by wind-pollination, as all of the species listed above are insect-pollinated. P-O ratios of *T. brevifolia* are of the same order of magnitude as those of other wind-pollinated species, even those with monoecious rather than dioecious breeding systems. For example, *T. canadensis* (Allison, personal communication) and two *Carya* species (McCarthy and Quinn 1990) all have P-O ratios in excess of 1 million. It would be informative to standardize methodologies and make more exact comparisons of P-O ratios of closely related species with different combinations of breeding systems and pollination syndromes.

Pollination Phenology

Variation in timing of pollen shedding occurred along a gradient of elevation and overstory canopy. The most commonly cited cue for flowering in woody perennials is the accumulation of hours above a certain threshold temperature

(heat sums) (Rathke and Lacey 1985). Heat sums decrease with elevation, and one may assume that heat sums are greater in the open than beneath overstory due to greater insolation (Collins *et al.* 1985).

The duration of pollen shedding tended to decrease with elevation and canopy closure, both for individual trees and for populations. Differences in duration of flowering among populations might have been due to environmental differences among sites, or to genetic differences among populations. Where differences existed between OPN and CAN populations within a site (*e.g.* LC1), the cause was more likely to be small-scale heterogeneous or ambiguous environmental cues. Extended flowering within an individual tree could have resulted from differential heat loads on individual branches due to self- shading. Self-shading was probably responsible in part for the later shedding date of the lowest branch compared to the highest branch surveyed on each tree. Another potentially important factor in determining length of shedding is damage due to environmental extremes. There was evidence of frost damage on open-grown trees, particularly at the two Higher Cascade sites (personal observation), and it is possible that slightly frost-damaged strobili suffered from delayed anthesis.

Phenological differences between sites, especially when comparing the Cascade Mountains to the Willamette Valley, might have been due in part to genetic differences resulting from differential selection pressures or genetic drift. For example, extended flowering times on a population level would result if early flowering were favored in some years and late flowering in others due to weather conditions

(Primack 1985). If weather conditions were less predictable in the Willamette Valley than the Cascade Mountains, greater genetic variation in phenological traits would be expected in the valley. Common garden studies would help clarify the importance of the genetic component of phenological variation in *T. brevifolia*.

Anthesis and receptivity of most of the female trees examined largely corresponded with male pollen shedding at each site. This suggests that female anthesis occurs in response to similar environmental cues as male pollen shedding. Some females also showed signs of receptivity (presence of pollination drop) after pollen shedding was no longer observed in the population. This 'long tail' of female receptivity may be indicative of 'bet hedging' (Stephenson 1981) in *T. brevifolia*. For example, if weather conditions caused pollination failure during peak pollen shedding some years, trees with asynchronous anthesis might experience greater reproductive success than trees with synchronous anthesis.

Phenology of Seed Development

Mature seed production was asynchronous both within and among *T. brevifolia* trees. Mature seeds were produced over a 12 to 15 week period at all study sites, and there were no obvious peaks of seed availability (Figure 2.5). Also, seeds from all stages of development could be found on most trees from the beginning of August into November and December (Figure 2.6; personal observation).

Two proximate mechanisms have been proposed to account for asynchronous ripening in *T. brevifolia*: *i.* development

is constant and maturation date varies due to variation in pollination date, and *ii.* the rate of development varies among seeds (Anonymous 1992a). The present study shows that the former mechanism cannot entirely account for variation in development rates because the majority of pollination occurs over 3 to 20 days while the maturation window lasts 12 to 15 weeks. It is probable that asynchrony in seed maturation is due to a combination of pollination date and development rate. Sites that began pollination earlier also reached 50% seed development earlier, but the proportion of seeds that developed by the end of the season in 1994 was highest for site HC2, which began pollination last in that year.

There are several possible explanations for the ultimate function of protracted seed maturation in *T. brevifolia*. Asynchronous fruiting could represent an adaptation to stochastic sources of seed loss (Rathke and Lacey 1985). For example, a seed predator might forage intensively on individual trees, removing most or all of the ripe fruit. Asynchronous ripening would prevent loss of the entire seed crop in one foraging event. Predators might also serve as important seed dispersers (which is probably the case for *T. brevifolia*, see Chapter 1). Prolonged ripening would force predators to move from tree to tree because there would never be large quantities of ripe seed available on any one tree. This would increases the chances for successful dispersal away from the parent (Howe and Smallwood 1982).

It is also possible that seed production is asynchronous in *T. brevifolia* because it is constrained to

be so. One common source of constraint for understory plants in resource limitation (Rathke and Lacey 1985), which precludes the synchronous production of a large quantity of seed (in the absence of resource storage). Competition among ovules for resources (Stephenson 1981) could result in variation in development rates within a plant (Primack 1985).

The presence of constraints on seed production is indicated by the failure of a proportion of seed to attain maturity on most trees in this study. In many cases, the seed integument remained green and the aril failed to fully form. The causes for this lack of maturation are not known. It appears that seed maturation is controlled by factors other than those controlling onset of pollination, because there was poor correspondence between the onset of pollination and proportion of mature seed present on bagged branches (Tables 2.6, 2.7). Immature seeds usually contained apparently normal embryos and megagametophytes. Excised embryos germinated *in vitro*, though at a lower rate than embryos from seed with fully formed arils (Vance, in preparation), although Flores *et al.* (1993) had conflicting results. Green seeds were not submitted to germination tests in the present study, but green undeveloped seeds from a *Taxus* cultivar have been found to have lower germination rates than seeds with fully formed arils (Heit 1969).

Seed Size and Developmental Phenology

T. brevifolia seed mass was negatively correlated with the elevation of the study site. Vance *et al.* (in preparation) found identical patterns in seed dimensions and

embryo size for seed collected from some of the same populations used in the present study. Melzack and Watts (1982) observed that seed mass of *T. baccata* decreased along a temperature and moisture gradient in England. This might be analogous to the decrease with elevation observed for *T. brevifolia*.

The negative correlation of seed mass with elevation is interesting in the light of the developmental phenology of the seed. Upper elevation sites generally had a shorter period in which to mature seed (based on later pollination dates) but a larger proportion of seed reached a mature state (with predators excluded) than in the VAL populations. Small seed size may represent an adaptation for higher elevation trees that compensates for a shorter growing season. Mature seed size may vary due to physiological effects of temperature on maturation, or may represent genetic differentiation along an elevational cline. Seed size does not appear to correlate with viability, as viability was lowest for the site with the largest seeds, a finding that was corroborated by embryo germination tests (Vance, in preparation).

Variation in Strobilus Production

There was little evidence of mast seeding behavior in the eight *T. brevifolia* populations over the three years of this study. Although female strobilus production showed some significant interannual variation, seed production was not significantly different among years. This does not rule out the possibility of masting in *T. brevifolia* populations. Other conifers have mast intervals of 5 to 10 years

(Schopmeyer 1974), and it is possible that the three years of this study were all poor seed crop years. Masting is hypothesized to be an adaptation to seed predation that results in predator satiation during mast years, and/or predator starvation in non-mast years (Silvertown 1980). In order for masting to be effective, the tree seed must constitute a significant portion of the diet of the predator (Silvertown 1980). In most situations *T. brevifolia* seed production is a fraction of that of dominant overstory conifers. Therefore, it is unlikely that masting would represent an effective adaptation to seed predation for *T. brevifolia* unless mast years were synchronous with those of overstory trees.

Interannual variation in strobilus production was small compared to variation among sites, among trees and within trees in a given year. The lack of correspondence of seed production and strobilus production in year-to-year differences might have been due to inherent limitations on the size of the seed crop *T. brevifolia* can produce (Chapter 4). Since these comparisons were done on unbagged branches, they also might reflect differences in predation rates among years. However, interannual comparisons of mean seed production on branches bagged to exclude predators showed identical patterns as unbagged branches (not shown).

The bulk of the variation in strobilus production observed in this study occurred among trees within a site and among branches within a tree. Tree to tree variation might be due to differences in microsite, genetic differences among trees, or chance. The rank ordering of the trees did not change significantly between 1993 and

1994, which argues against the influence of chance (the lack of significant correspondence with 1992 ranking may be due to low statistical power resulting from small sample sizes). In order to partially distinguish between environmental and genetic effects, one can search for environmental factors that correlate with strobilus production. In th0is study, overstory canopy and its effect on the light environment of the tree was identified as one of the primary external factors likely to affect reproductive output of the trees. Chapter 3 presents a detailed analysis of the relationship of overstory with reproductive potential, and Chapter 4 examines factors affecting seed production.

CHAPTER 3 GROWTH AND REPRODUCTIVE POTENTIAL OF *TAXUS BREVIFOLIA* UNDER A RANGE OF OVERSTORY CONDITIONS IN WESTERN OREGON

INTRODUCTION

The amount of overstory canopy cover is a predominant factor determining the growth and reproduction of most understory species. Overstory removal typically results in elevated light levels and increased availability of other resources such as water or nutrients (Horn 1971). Understory plants are often 'released' by overstory removal, and growth rates increase considerably (Canham and Marks 1985). Flower and seed production of many understory species is limited by shading (Bunnell 1990; Dale and Causton 1992B; Devlin 1988; Hicks and Hustin 1989; Lee 1989; Niesenbaum 1993), and many understory plants achieve successful sexual reproduction only in elevated light levels found beneath overstory gaps (Thompson and Willson 1978).

One way plants adjust to changes in illumination is by altering the amount of resources devoted to photosynthetic and support structures. Shaded plants tend to maximize photosynthetic surface area while minimizing investment in leaf tissue. Thus foliage-to-branch ratios tend to be higher in shaded plants (Niesenbaum 1993) and specific leaf area (the ratio of leaf area:leaf weight) is often inversely proportional to light levels (e.g. Dale and Causton 1992a; Hughes 1965; Jones and McLeod 1990; Tucker *et al.* 1987). This is because leaves of shaded plants are less dense than those of open-grown plants (Beets and Lane 1987). Branch morphology can also vary greatly with illumination levels. Trees growing in shaded environments tend to

produce monolayers of branches with horizontal leaf presentation, which minimizes self-shading (Horn 1971; O'Connell and Kelty 1994). Branch bifurcation ratios (the ratio of the number of distal to proximal branches, Veres and Picket 1982) also vary in response to light levels. For example, branches of *Acer saccharum* (Steingraeber *et al.* 1979) and *Lindera benzoin* (Veres and Pickett 1982) both showed lower bifurcation ratios under shade than in open areas, which indicates more secondary branching in the open.

The present study explores the effect of overstory removal on *Taxus brevifolia* Nutt. (Pacific yew), an understory conifer of the Pacific northwest of North America. *T. brevifolia* has received much recent attention because its bark was discovered to contain a potent anti-cancer compound (Wani *et al.* 1971). Hundreds of thousands of *T. brevifolia* trees have been destroyed in the United States and Canada to provide raw material for Taxol extraction, with unknown effects on the viability of *T. brevifolia* populations. The present study was part of a larger effort to assess the impact of management and harvesting activities on natural *T. brevifolia* populations.

T. brevifolia occurs most frequently in the understory of late seral forests of the Pacific Northwest (Busing *et al.* 1995; McCune and Allen 1985). However, *T. brevifolia* is not an obligate understory species, and it tolerates a broad range of overstory canopy coverage (Bolsinger and Jaramillo 1990; Busing *et al.* 1995). Important limitations on *T. brevifolia* occurrence are probably its high susceptibility to fire (Crawford 1983; Stickney 1980) and relatively low rate seed production, dispersal, and/or establishment

relative to other coniferous trees (Busing *et al.* 1995; McCune and Allen 1985). If flowering of *T. brevifolia* is restricted by low light levels, then overstory gaps could be of crucial importance in the tree's life cycle.

Partial harvesting of overstory trees could become commonplace under 'New Forestry' practices and ecosystem management (Franklin 1989), and large numbers of surviving *T. brevifolia* trees could be exposed to open canopy conditions. In order to devise effective management strategies and ensure the viability of *T. brevifolia* populations, it is necessary to document the response of *T. brevifolia* to overstory removal. Previous work showed that *T. brevifolia* responds strongly to overstory removal. Crawford (1983) found that 2 years after a clearcut harvest of overstory trees in Idaho, *T. brevifolia* trees showed increased branching, increased epicormic shoots, shorter shoots and needles, increased needle deflection and decreased seed production as compared to control trees with no overstory canopy harvest. Bailey and Liegel (unpublished) found that *T. brevifolia* showed increased diameter growth following partial overstory removal, as compared to nearby control trees under relatively intact canopy. Kelsey and Vance (1992) found that *T. brevifolia* trees in a clearcut had higher sapwood to heartwood ratio, thicker bark, lower specific leaf area (ratio of needle area to mass) and lower taxane concentrations in bark tissue than trees under intact canopy. Allison (1991) found that *Taxus canadensis* showed higher seed production in canopy gaps compared to nearby plants under full canopy.

The main focus of the present study was to determine the relationship between overstory canopy and reproductive potential of wild *T. brevifolia* populations in western Oregon. Also, in order to determine if overstory removal resulted in reduced tree vigor, as had been reported previously for *T. brevifolia* (Anonymous 1992a; Crawford 1983), the relationship between overstory canopy and average 5-year growth increment and the mass of new growth on branches is examined. Finally, in order to gain insight into the modes of adaptation to changing light levels the association between overstory canopy and specific leaf area, foliage-to-branch mass ratios, and branch bifurcations is examined. The study was carried out on four geographically and vegetationally distinct sites over two years in order to assess the generality of the relationships.

METHODS

Study Design and Tree Selection

This study was conducted on 4 sites in western Oregon (Table 2.1). The main criteria for site selection were the presence of at least 10 sexually mature *T. brevifolia* trees per hectare and a range of canopy cover above the *T. brevifolia* trees. Working systematically from an arbitrary starting point, all *T. brevifolia* trees greater than 3 m tall and 5 cm basal diameter were mapped until at least 30 male and 30 female trees were included. Trees separated by 2 m or less were considered ramets of the same genet, and were mapped as one tree. None of the trees selected for intensive study were within 3 m of one another.

In order to ensure an adequate range of overstory canopy cover, trees were selected by stratified random sampling. Trees were subjectively assigned to two groups: those growing under open canopy (OPN) and those under canopy cover (CAN). 7 male and 7 female trees were randomly selected from each canopy grouping, for a total of 28 trees per site. This selection technique resulted in two spatially separated populations per site, as described below. For convenience, 'site' will refer to an entire drainage and 'plot' will refer to a canopy grouping within a site.

Study Sites

The Higher Cascade 1 site (HC1) was located in the Snow Creek drainage of the Willamette National Forest (44° 23' 30" N; 122° 15' W). Overstory trees in the OPN plot were harvested in a 1990 clearcut. The CAN plot was located approximately 1 km upslope from the OPN plot.

The Higher Cascade 2 site (HC2) was located in the Hackleman Creek drainage of the Willamette National Forest (44° 24' N; 122° 02' W). Overstory trees for the OPN plot were harvested in a 1978 salvage logging operation, and substantial windthrow of remaining trees had occurred since that time. The CAN plot was located approximately 1.5 km downslope from the OPN plot.

The Low Cascade site (LC) was located in the McCrae Creek drainage of the H.J. Andrews Experimental Forest (44° 15' N; 122° 11' W). Canopy gaps in the OPN plot were

created by a 1981 clearcut and construction of a road in the 1950's. The CAN plot was contiguous with the OPN plot.

The Willamette Valley site (VAL) was located in the west fork of the Oak Creek drainage of the McDonald Research Forest in the foothills of the Oregon Coast Range (44° 35' N; 123° 35' W). Overstory trees in the OPN plot were harvested in a 1984 clearcut. The CAN plot was located approximately 2 km downslope from the OPN plot.

More detailed information about study sites is provided in Chapter 2.

Male Strobilus Production

Strobilus production was determined on subsampled branches, and no attempt was made to estimate the productivity of entire trees. This method should reveal trends that might otherwise have been obscured by errors in estimates of whole tree productivity.

A pilot study was performed in 1992 on a subset of the trees used in 1993 and 1994. Ten branches were selected on each tree, clipped, and transported to the lab where strobili were counted.

In 1993 8 branch tips were selected on each tree by stratified random sampling. Each tree canopy was first divided into upper and lower sectors and 4 branches were randomly selected from each sector. Beginning at the basipetal portion of the branch, successive bifurcations were randomly chosen until arriving at a branch apex. Branches were measured 20 cm basipetal from the selected apex and marked with nylon flagging. All branch tips were sampled acropetal to the 20 cm mark. Strobili were counted

in April and categorized as 'viable' and 'nonviable' based on color, external morphology and size. These designations were confirmed by following the fates of individual strobili.

In April of 1994 new branch apices were selected to compensate for mortality (≈1% of all branches). Branches were clipped at the 1993 flagging and transported to the lab in separate paper bags. Branches were remeasured 20 cm basipetal from the apex, clipped, and all branch material between 20 cm and the 1993 flagging was discarded. The number of strobili on these 20 cm branch tips were counted and categorized as 'viable' and 'nonviable.' Stems and foliage were weighed after drying at 65° C for 48 hours.

Ovule Production

A pilot study was performed in 1992 on a subset of the trees used in 1993 and 1994. 4 branches were selected per tree, 30 cm was marked basipetal from the apex, and ovules were counted in May, June and July.

In 1993, 8 branch apices were selected on female trees using the protocol described for male trees, except 30 cm branch segments were sampled instead of 20 cm, because female strobilus production was much lower than male strobilus production (Chapter 2). The number of visible ovules was determined in May, June and July. The maximum of the three counts was used as the estimate of ovule production.

1994 ovule production was measured in much the same way as in 1993. In late September of 1994 all branches were clipped at the 1994 flagging. Branches were clipped in the

fall rather than in the spring because seed development and attrition were followed for a related study (Chapter 4). All growth distal to 1994 bud scars was clipped and separated. All branch material was dried at 65° C for 48 hours and stems, foliage and 1994 growth were weighed separately.

Specific Leaf Area

Specific leaf area (SLA) was estimated for female trees in 1994. The two branches with highest and lowest strobilus production were chosen for each tree. The first 10 fully expanded needles were selected from 1993 growth and projected leaf area was measured with an imaging system (Campbell 1990). Needles were dried and weighed, and SLA was calculated as twice the projected leaf area of 10 needles divided by needle dry weight. The variable used for this study was the average SLA of the two branches.

Increment Coring

To determine ages and growth rates of the study trees, an increment core was extracted 30 cm above the base on the north face of each tree. Cores were analyzed under a stereo microscope (30x) using a razor blade to expose a clean, smooth surface from which increments could be easily discerned. The total number of increments were counted and the width of the first five full increments proximal from the cambium was measured. In some cases age had to be estimated because some increments were obliterated by heartwood rot.

Tree Size

Tree size was estimated by measuring the height (h) of the stem up to 1 cm diameter, and diameter at breast height, 1.37 m (dbh). A combination of these variables, $(dbh)^2 \cdot h$, was previously shown to be linearly related to total aboveground biomass of *T. brevifolia* (Russell 1973).

Potential Solar Radiation

Annual potential solar radiation was determined for each tree by interpolating among values for slope, aspect and latitude in the tables of Buffo *et al.* (1972). This is essentially an estimate of the cumulative amount of solar radiation incident on the overstory, in $cal/cm^2/year$. The amount actually reaching the *T. brevifolia* trees would be a function of overstory openness.

Slope was measured at each tree by siting with a clinometer from 10 m above to 10 m below each tree. Aspect was measured with a compass, siting in the same direction as the clinometer.

Overstory Canopy

Overstory canopy openness above and to the south of each study tree was estimated using the model LAI-2000 plant canopy analyzer (LI-COR, Lincoln, NE, USA). This instrument derives estimates of canopy cover from measurements of diffuse radiation. A refernce sensor placed in a clearing automatically logged light readings every 30 s while readings were taken above each *T. brevifolia* tree with a measurement sensor. Both sensors were levelled and pointed in exactly the same direction. Because of the difficulty in finding sufficiently large open areas near the *T. brevifolia*

populations, a 270° lens cap was used on both sensors. Measurements were taken in a southwesterly direction between 7 am and 11 am and in a southeasterly direction between 4 pm and 8 pm, because direct sunlight confounds readings. All measurements were taken under relatively uniform sky conditions, usually on cloudless days. Most measurements were taken during July and August of 1993.

The value used in this study was Diffuse Noninterceptance (DIFN), an estimate of the amount of sky visible from beneath the canopy. DIFN ranges from 0 (completely closed canopy) to 1 (no canopy) (Welles and Norman 1991). The average of the southwest and southeast DIFN readings is an estimate of the openness of the canopy directly above and surrounding the southern aspect of each *T. brevifolia* tree. The southern aspect was measured on the assumption that this was the location of the most important canopy gaps at this latitude (i.e. those that resulted in the most significant increases in understory light levels).

Analysis

Rationale

The measurement of overstory openness was not a direct quantification of the level of photosynthetically active radiation (PAR) available to the *T. brevifolia* trees. The amount of PAR reaching the understory is also a function of the slope and aspect of the terrain. In order to account for this effect, potential radiation was used as an explanatory variable along with DIFN in analyzing the factors associated with *T. brevifolia* growth and reproduction. Another way this problem was approached was

by using specific leaf area as a sort of 'phytometer' of light incident on the foliage, since SLA is inversely proportional to light under controlled conditions for many plant species (Dale and Causton 1992a; Hughes 1965; Jones and McLeod 1990).

Other variables included in the analyses were the mass of the sampled branches, the ratio of foliage to total mass of sampled branches, the mass of 1994 branch growth, and the age and size of the trees. Each of these variables could affect the magnitude or intensity of strobilus production or growth. Branch mass could have a direct effect because greater mass suggests larger branches and more physical area for reproductive buds to form on. Reproductive effort and growth often vary with the age and size of plants. For example, reproductive effort is often negatively correlated with plant size within a species (Samson and Werk 1986), and middle-aged trees often produce more seeds than very young or very old trees (Kozlowski 1971). The ratio of foliage to branch mass might be an indicator of branch vigor, as senescing branches are likely to abscise foliage (Kozlowski 1971). Finally, the average mass of 1994 branch growth might indicate the vigor of the selected branches, or overall tree vigor.

Statistics

The multiple linear regression procedure of SAS (SAS Institute 1987) was used to assess the relationships between overstory openness and other explanatory variables and strobilus production, growth and morphology. Variables were transformed where appropriate to correct for

heteroscedasticity. The variable selection technique involved adding or dropping terms from the model and assessing the change in the regression sum of squares relative to the change in degrees of freedom. Final models were the most parsimonious models that yielded an F-statistic greater than expected at the 95% confidence level when compared to the next simplest model (Neter and Wasserman 1974).

Several explanatory variables exhibited multicollinearity, as indicated by a matrix of correlation coefficients (Appendix B). To account for this, each analysis was performed in three phases. The first phase was a backward selection procedure with DIFN and all variables that showed weak multicollinearity as explanatory variables, including interactions with site indicator variables. All variables that significantly improved the fit of the regression were retained in the model. The second phase was a forward selection procedure with the final model from phase one as a starting point. Variables that showed multicollinearity with DIFN were tested individually in this phase. Sites were again treated separately by including interaction terms with site indicator variables. In the final phase, regression lines were tested for equality by systematically combining indicator variables and testing interaction terms versus the full model with separate slopes and intercepts. The goal of this phase was to determine the generality of the relationships between the explanatory variables and the response, and to assess the impact of site differences on those relationships.

RESULTS

Reproductive Potential

General Patterns

Overstory openness was the variable most consistently associated with male and female strobilus production in both years of the study. The backward stepwise regressions indicated that even when taking the effects of size and age of the trees into account, overstory openness explained a significant amount of the variation in ovule and male strobilus production (Table 3.1). Also, the forward stepwise regressions indicated that the variables average branch weight, potential radiation, specific leaf area and dry mass of 1994 branch growth did not account for significant variation in ovule and strobilus production beyond that explained by DIFN (Table 3.1). Finally, $DIFN^2$ did not significantly improve the fit of the lines, indicating that the relationship between DIFN and the natural log of ovule and strobilus production was not significantly nonlinear.

Male Strobilus Production

In 1993 DIFN and the ratio of foliage to branch weight were significantly associated with male strobilus production (Tables 3.1, 3.2; Figure 3.1A). The relationship was nearly identical when only viable strobili were used as a response (Table 3.2; Figure 3.1C). The regression lines for the two high elevation sites were not significantly different from each other, but differed from the LC and VAL lines. Branch productivity was higher on average for the higher Cascade

Table 3.1 Summary of results of stepwise regressions. Values in table are the probability of a larger F-statistic under the null hypothesis that the variable does not explain significant variation in the data. In 'Backward' procedure, each variable was tested with all other variables in the model. In 'Forward' procedure, each variable was tested against a model containing DIFN. At this stage of the analysis, all variables were tested with interactions with site variables included, so four terms (separate slopes for each site) were added or dropped for each variable. All response variables were transformed by natural logarithms to correct for heteroscedasticity. ♂STROB- average number of male strobili/20 cm branch segment; OVULE- average number of ovules/30 cm branch segment; 94GRTH- dry mass of branch material distal to 1994 bud scars (g); 5YRINCR- width of 5 year increment (mm); SIZE- approximation of tree size ($d^2 \bullet h$)(m^3); AGE-number of increments; DIFN- overstory canopy openness; BRWT- average dry mass of branch samples (20 cm for males, 30 cm for females)(g); POTRAD- potential solar radiation ($cal/cm^2/yr$); FOLBR- ratio of mass of foliage to BRWT; SLA- specific leaf area (cm^2/g); NA- analysis not performed for that variable.

	♂STROB93	♂STROB94	OVULE93	OVULE94	94GRTH	5YRINCR
			Backward			
SIZE	0.17	0.72	0.13	0.23	0.20	0.79
AGE	0.60	0.89	0.51	0.86	0.76	0.0047
DIFN	<0.0001	<0.0001	0.00002	0.01	<0.00001	<0.00001
			Forward			
BRWT	0.24	0.97	0.44	0.13	0.0001	NA
POTRAD	0.24	0.65	0.92	0.95	0.20	0.13
FOLBR	0.00009	0.08	0.48	0.93	0.29	NA
SLA	NA	NA	0.62	0.24	0.45	NA
94GRTH	NA	NA	0.41	0.40	–	NA
$DIFN^2$	0.37	0.95	0.67	0.09	0.19	0.35

sites at high canopy closure than at LC or VAL, but branch productivity converged for the VAL and high elevation sites under open canopy conditions (Table 3.2; Figure 3.1A,C).

1994 strobilus production was also significantly associated with DIFN, but none of the other explanatory variables accounted for significant additional variation (Table 3.1). The intercepts differed between the high elevation sites and the LC and VAL sites, which were not

significantly different. The slopes were not significantly different among the four sites. As in 1993, the high elevation sites had higher strobilus production than the lower elevation sites (Table 3.2; Figure 3.1B). When damaged strobili were excluded from the analysis, there was no longer a significant relationship between DIFN and 1994 strobilus production at the HC1 site (Table 3.2; Figure 3.1D).

Ovule Production

Overstory openness was significantly and positively associated with ovule production in 1993 at all sites, and none of the other variables tested accounted for significant variation in ovule production beyond that explained by DIFN (Table 3.1). The regression line for the higher Cascade sites was significantly different from that of VAL and LC, and ovule production was higher on average for the higher Cascade sites than for the 2 lower sites, although the fitted lines nearly converge under open canopy (Table 3.2; Figure 3.1E). In 1994, ovule production was also positively associated with overstory at sites HC2, LC and VAL, but not at HC1 (Table 3.2; Figure 3.1F). Again, the higher Cascade sites had higher average strobilus production than VAL and LC under heavy canopy cover, and ovule production at the four sites tended to converge under open canopy.

Correlations With 1992 Reproductive Potential

In order to test the predictive power of the regression equations, predicted values generated by the male strobilus production and ovule production equations were correlated with data collected in 1992 on a subset of the trees used

Table 3.2 Regression equations for reproductive potential versus overstory openness (DIFN) and the ratio of mass of foliage to dry mass of branches (FOL/BRWT). STROB- average number of male strobili produced per 20 cm branch segment; SHED- average number of viable male strobili produced per 20 cm branch segment; OVPROD- average number of ovules produced per 30 cm branch segment. Averages were based on 8 branch samples per tree. Year of measurement follows variable name. X_i- indicator variable for site i; 1=High Cascade 1, 2=High Cascade 2 and 3=Low Cascade and 4=Willamette Valley. If more than one subscript appears then the parameter estimate was not significantly different for those sites. df- total degrees of freedom. Standard errors for estimates of regression parameters are in parentheses below each estimate. ns- estimate not significantly different from 0 at $p \leq 0.05$. All other abbreviations are as described in Table 3.1.

Model	df	R^2
MALES		
$LN(STROB93) = -1.55 \cdot X_{12} - 2.66 \cdot X_3 - 2.75 \cdot X_4 + 1.94 \cdot DIFN \cdot X_{123} + 3.14 \cdot DIFN \cdot X_4 + 6.39 \cdot FOL/BRWT$ $(0.84)^{ns} \quad (0.83) \quad (0.81) \quad (0.25) \quad (0.31) \quad (1.21)$	67	0.81
$LN(SHED93) = -1.58 \cdot X_{12} - 2.75 \cdot X_3 - 2.84 \cdot X_4 + 1.89 \cdot DIFN \cdot X_{123} + 3.23 \cdot DIFN \cdot X_4 + 6.34 \cdot FOL/BRWT$ $(0.85)^{ns} \quad (0.84) \quad (0.82) \quad (0.25) \quad (0.32) \quad (1.23)$	67	0.81
$LN(STROB94) = 2.38 \cdot X_{12} + 1.79 \cdot X_{34} + 2.57 \cdot DIFN$ $(0.16) \quad (0.14) \quad (0.26)$	65	0.64
$LN(SHED94) = 2.24 \cdot X_1 + 1.93 \cdot X_2 + 0.75 \cdot X_{34} + 0.84 \cdot DIFN \cdot X_1 + 3.18 \cdot DIFN \cdot X_{234}$ $(0.35) \quad (0.21) \quad (0.16) \quad (0.58)^{ns} \quad (0.32)$	65	0.65
FEMALES		
$LN(OVPROD93) = 2.50 \cdot X_{12} + 0.98 \cdot X_{34} + 1.09 \cdot DIFN \cdot X_{12} + 2.33 \cdot DIFN \cdot X_{34}$ $(0.19) \quad (0.24) \quad (0.38) \quad (0.44)$	50	0.54
$LN(OVPROD94) = 2.47 \cdot X_1 + 1.19 \cdot X_2 - 0.015 \cdot X_{34} + 0.29 \cdot DIFN \cdot X_1 + 3.52 \cdot DIFN \cdot X_{234}$ $(0.41) \quad (0.26) \quad (0.26)^{ns} \quad (0.64)^{ns} \quad (0.49)$	50	0.56

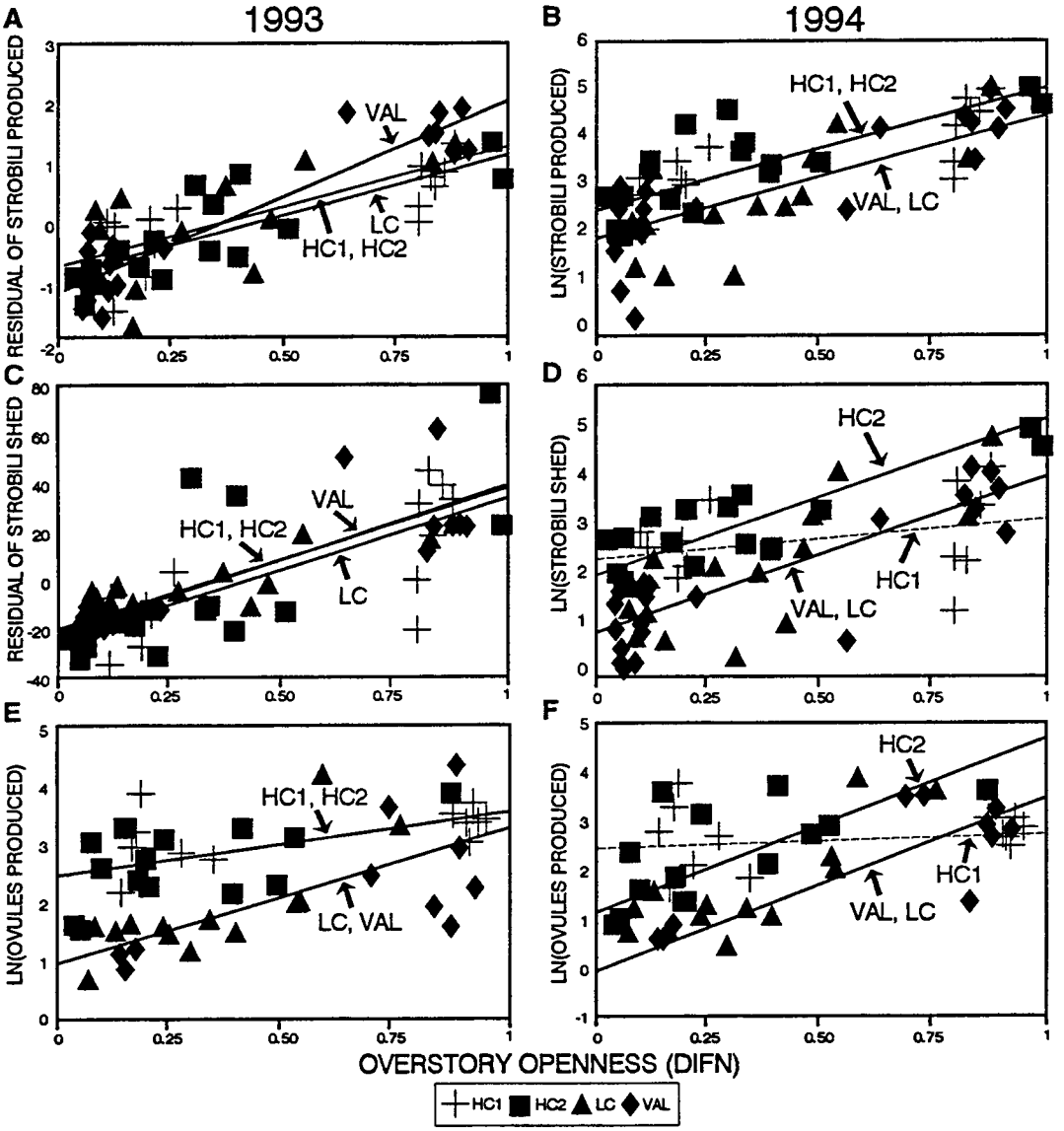

Figure 3.1 Regressions of strobilus production versus overstory openness (DIFN). Each point is an observation from a single tree. Symbols indicate sites. **A.** Components effects plot with overstory openness versus residuals from regression of average number of male strobili per 20 cm branch segment versus the ratio of foliage to branch weight. **B.** Average number of male strobili per 20 cm branch segment, 1994. **C.** Component effects plot with overstory openness versus residuals from regression of average average number of male strobili per 20 cm branch segment in 1993 versus the ratio of foliage to branch weight. **D.** Average number of viable male strobili per 20 cm branch segment, 1994. **E.** Average number of ovules per 30 cm branch segment, 1993. **F.** Average number of ovules per 30 cm branch segment, 1994. Broken lines have slopes that are not significantly different from 0. Regression equations and R^2 values are provided in Table 3.2.

Table 3.3 Pearson's correlation coefficients for predicted values from 1993 and 1994 regression equations (Table 3.2) and 1992 reproductive potential. ♂STROB92- 1992 male strobilus production; OVPROD92- number of ovules produced/30 cm branch segment, 1992. $p \leq 0.0001$ for all coefficients.

	♂STROB92	OVPROD92
N	20	28
Predicted Values, 1993	0.78	0.78
Predicted Values, 1994	0.63	0.85

in 1993 and 1994. The correlations were positive and highly significant in all four cases (Table 3.3).

Growth

The width of 5-year increment was positively associated with overstory openness and negatively associated with age. The regression line for sites HC1, HC2 and LC was significantly different from the line for VAL (Table 3.4; Figure 3.2A). The equation accounts for nearly half of the observed variation in increment growth (R^2=0.46, Table 3.4).

Dry mass of 1994 growth on female branches was positively associated with DIFN at the VAL and HC1 sites, but not at the HC2 and LC sites. Trees at the VAL site had the lowest growth under shaded conditions, and the highest growth under open conditions. HC1 was second lowest in the shade and second highest in the sun (Table 3.4; Figure 3.2B). Overstory openness accounted for over half of the

Table 3.4 Regression equations for growth and morphological characteristics versus overstory openness (DIFN). 5YRINCR- width of 5 year increment (mm), male and female trees; 94GRTH- average dry mass of 1994 growth (g), female trees; SLA- average specific leaf area (cm^2/g), female trees; BIFUR- average # bifurcations per 30 cm branch, female trees; BRWT- average dry mass of sampled branch segments, measured in early spring for males and late summer for females; FOL/BRWT- average ratio of dry mass of foliage to BRWT. Transformations corrected for heteroscedasticity. Standard errors for estimates of regression parameters are in parentheses below each estimate. ns- estimate not significantly different from 0 at $p \leq 0.05$. All other abbreviations are as described in Table 3.1.

Model	df	R^2
$LN(5YRINCR) = \underset{(0.23)}{1.32 \cdot X_{123}} + \underset{(0.40)}{1.58 \cdot X_4} + \underset{(0.29)}{1.11 \cdot DIFN \cdot X_{123}} + \underset{(0.28)}{2.47 \cdot DIFN \cdot X_4} - \underset{(0.0015)}{0.0035 \cdot AGE \cdot X_{123}} - \underset{(0.005)}{0.014 \cdot AGE \cdot X_4}$	112	0.46
$LN(94GRTH) = \underset{(0.24)}{-0.97 \cdot X_1} - \underset{(0.16)}{0.414 \cdot X_2} - \underset{(0.18)}{0.81 \cdot X_3} - \underset{(0.23)}{1.27 \cdot X_4} + \underset{(0.36)}{1.40 \cdot DIFN \cdot X_1} + \underset{(0.41)^{ns}}{0.63 \cdot DIFN \cdot X_{23}} + \underset{(0.37)}{2.22 \cdot DIFN \cdot X_4}$	56	0.56
$1/SLA = \underset{(0.00037)}{0.00832} + \underset{(0.00070)}{0.003566 \cdot DIFN}$	51	0.33
$LN(BIFUR) = \underset{(0.08)}{2.35} + \underset{(0.15)}{0.98 \cdot DIFN}$	57	0.43
$LN(\sigma BRWT) = \underset{(0.059)}{0.13 \cdot X_{13}} + \underset{(0.05)}{0.35 \cdot X_{24}} + \underset{(0.09)}{1.26 \cdot DIFN}$	67	0.28
$LN(\varphi BRWT) = \underset{(0.08)}{1.29 \cdot X_{13}} + \underset{(0.08)}{1.63 \cdot X_2} + \underset{(0.11)}{1.07 \cdot X_4} + \underset{(0.13)}{1.13 \cdot DIFN}$	56	0.31
$\sigma FOL/BRWT = \underset{(0.011)}{0.66 \cdot X_{134}} + \underset{(0.015)}{0.70 \cdot X_2} - \underset{(0.021)^{ns}}{0.01 \cdot DIFN}$	67	0.21
$\varphi FOL/BRWT = \underset{(0.015)}{0.41 \cdot X_{134}} + \underset{(0.016)}{0.34 \cdot X_2} + \underset{(0.026)^{ns}}{0.027 \cdot DIFN}$	56	0.05

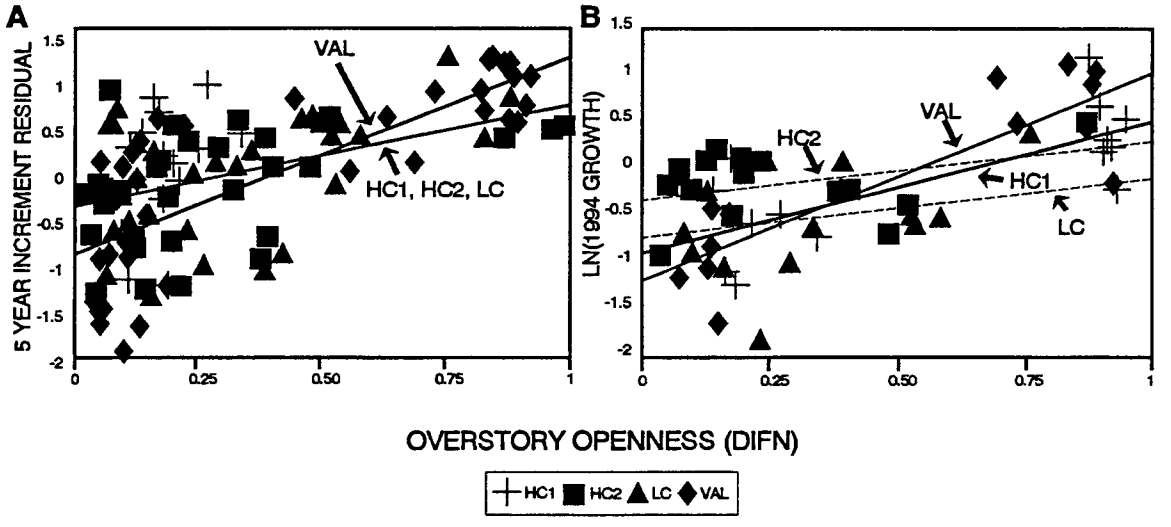

Figure 3.2 Regressions of growth versus overstory openness. **A.** Components effects plot with residuals from the regression of the width of 5-year increments versus age. **B.** Dry mass of 1994 growth on female branches. Broken lines have slopes that are not significantly different from 0. Regression equations and R^2 values are provided in Table 3.4.

variation in dry mass of 1994 branch growth (R^2=0.56, Table 3.4).

As for the analyses of reproductive potential per branch unit, the variables tree size, potential radiation, and $DIFN^2$ failed to significantly improve the models. In addition, for 1994 branch growth the ratio of foliage to branch weight and specific leaf area were not significant (Table 3.1). Branch weight was strongly associated with 1994 growth, but it was also correlated with DIFN, and obscured the relationship between DIFN and branch growth when included in the model. Therefore, branch weight was not included in the model presented in Table 3.4 and Figure 3.2B.

Morphology

For female trees, specific leaf area was inversely associated with DIFN (R^2=0.33; Table 3.4; Figure 3.3A) and the average number of bifurcations per branch was positively associated with DIFN (R^2=0.42; Table 3.4; Figure 3.3B). The relationships were not significantly different among the four sites.

The dry weight of sampled branches was significantly and positively associated with DIFN for male and female trees. For male trees, the regression line for HC2 and VAL differed from that of HC1 and LC, though the lines shared a common slope (Table 3.4; Figure 3.3C). For females the regression line of HC1 and LC differed from that of VAL and of HC2, although all three lines shared a common slope (Table 3.4; Figure 3.3D). The regression equations accounted for a relatively small proportion of the total variation in branch dry weight (R^2=0.28 and 0.31, respectively, Table 3.4).

The ratio of foliage to total branch dry weight was not significantly associated with overstory canopy for male or female trees, though there were significant differences between HC2 and the other 3 sites for this variable (Table 3.4; Figure 3.3E,F).

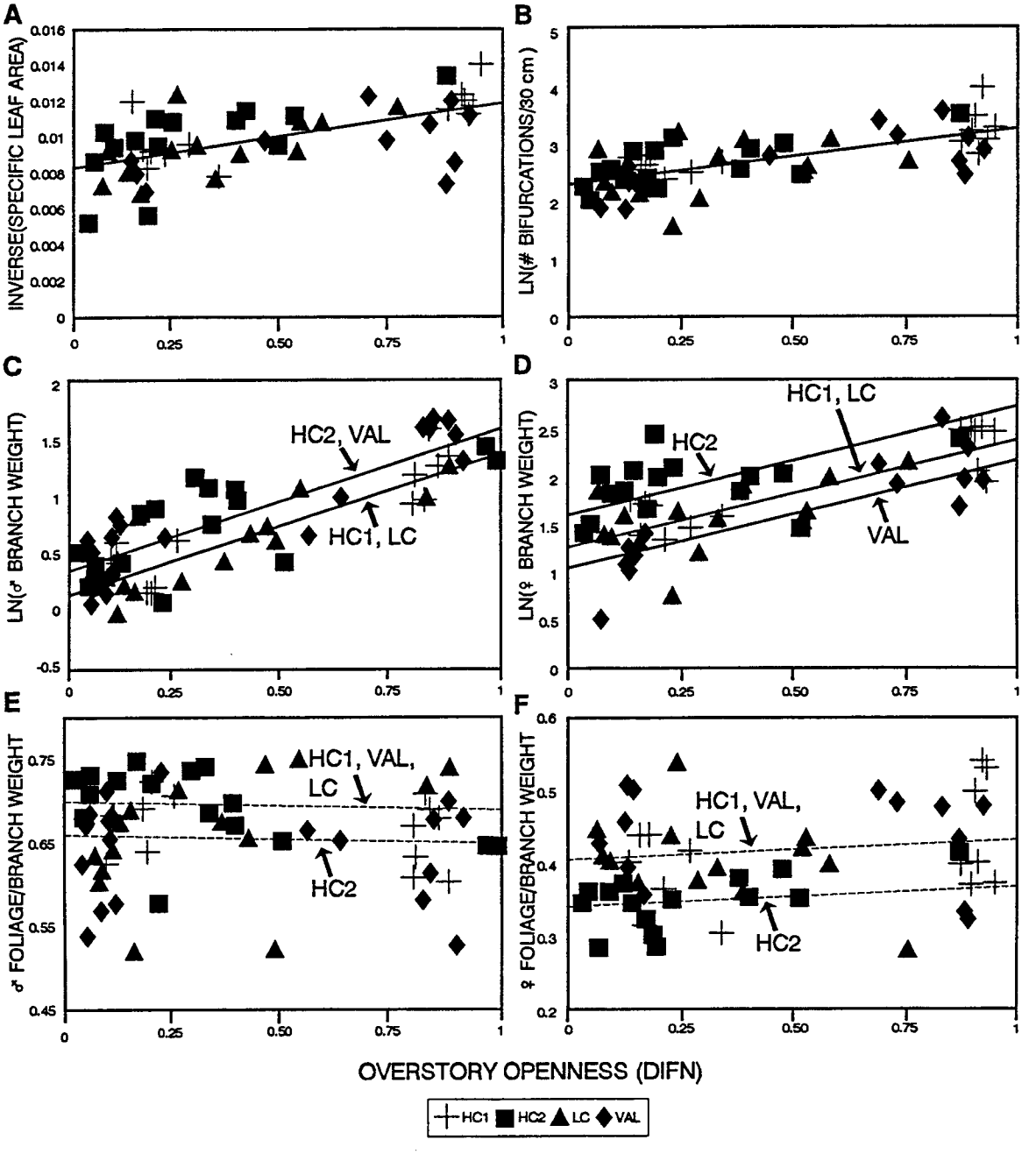

Figure 3.3 Regressions of morphological variables on overstory openness. **A.** Specific leaf area (cm^2/g), female trees. **B.** Number of bifurcations/30 cm branch segment, female trees. **C.** Average mass of 20 cm branch segment, male trees. **D.** Average mass of 20 cm branch segment, female trees. **E.** Ratio of dry mass of foliage to total dry mass of 20 cm branch segment, male trees. **F.** Ratio of dry mass of foliage to total dry mass of 30 cm branch segment, female trees. Broken lines have slopes that are not significantly different from 0. Regression equations and R^2 values are provided in Table 3.4.

DISCUSSION

Reproductive Potential

Reproductive potential of branch segments on *T. brevifolia* trees was strongly related to overstory openness. The positive relationship between DIFN and reproductive potential was consistent at four different sites over two growing seasons. In addition, most of the other variables tested, including tree size and age, mass of branch samples, potential radiation, specific leaf area, mass of 1994 branch growth and $DIFN^2$ did not significantly improve the fit of the regressions. The predictive power of the final regression equations is illustrated by the strong correlation between predicted values generated by these equations and the 1992 data set, which was not used to generate the equations.

However, the regression equations did not account for all of the variation in branch reproductive potential, as illustrated by the modest coefficients of variation (R^2 ranged from 0.54 to 0.81, Table 3.3). The regression lines differed for the different sites as well, suggesting that environmental factors other than those measured were also associated with branch reproductive potential.

The major exception to the relationship between DIFN and branch reproductive potential was female strobilus production in 1994 at site HC1. Part of the reason for this exception may have been stressful conditions on the clearcut at HC1 during the winter of 1994. Viable male strobilus production was also unrelated to overstory at this site in 1994. There was morphological evidence of frost damage to

male strobili in that year (personal observation), suggesting that frost also destroyed female strobili prior to anthesis.

The only other variable that accounted for significant variation in strobilus production in addition to that explained by DIFN was the ratio of foliage to branch dry mass (FOLBR) for 1993 male strobilus production. FOLBR was measured in the spring of 1994 for male trees. It may be that this variable acted as an indicator of branch vigor at the time of strobilus production. *T. brevifolia* branches typically abscise foliage during annual summer drought in western Oregon, and some branches lose all but the current and previous year's foliage (personal observation). Perhaps conditions were such in 1992 during the production of 1993 strobili that extensive foliage abscission and initiation failure occurred for some trees. 1992 was the driest year of the study (Oregon Climate Service, unpublished data), and moisture levels may have been associated with FOLBR and strobilus production in that year. FOLBR was not significantly associated with 1993 ovule production, perhaps because female branches were harvested at the end of 1994 and any effects from the 1992 season might have been obscured by 1994 growth. Another possibility is that production of male strobili was more sensitive to growing conditions than female strobilus production, because of different physiological controls on the process.

The results of this study contrast somewhat with those of related studies. Allison (1991) found that ovule production was higher and male strobilus production was unchanged on *T. canadensis* shrubs growing in a gap, compared

to nearby plants under full canopy. A possible explanation for this discrepancy is that the monoecious habit of *T. canadensis* allows competition between male and female strobili for resources, and that female strobili are a stronger sink for photosynthates. Other studies have also demonstrated a stronger response of female reproductive structures to light than male reproductive structures (Niesenbaum 1992; Silen 1973). In contrast, the relationship between overstory canopy and strobilus production was approximately the same for male and female strobili in the present study. In fact, there was little evidence of sex dimorphism in the trees examined in this study (unpublished data). Perhaps some differences between the sexes would have been detected if more intensely shaded trees had been included, as differences are more likely to be manifested under stressful conditions.

T. brevifolia trees in Idaho showed reduced seed production 2 years after overstory removal compared to control trees under intact overstory (Crawford 1983). It is possible that the trees were still suffering stress from being exposed to full sunlight. *T. brevifolia* may require several years to equilibrate following overstory removal. An alternative explanation is that ovule production of the Idaho trees actually was higher in the clearcut than in the control population, but that ovule attrition was higher for the clearcut. This would agree more closely with the pattern observed in the present study, where ovule production was elevated under open conditions, even two years after overstory removal, but mature seed production

was unrelated to overstory due to higher seed attrition under open conditions (Chapter 4).

Growth

Overstory openness was positively associated with tree growth, as indexed by 5-year increment for all trees and 1994 branch growth on female trees. 5-year increment was also negatively associated with tree age. This association may indicate an allometric relationship, or that older *T. brevifolia* trees grow more slowly than young trees. The fact that tree size was not significantly associated with 5-year increment argues against the former possibility (Table 3.1). Also, basal diameter of the trees did not significantly improve the fit of the 5-year increment regression equation containing DIFN ($F=1.08$, $p=0.37$, equation not shown).

The results of this study are in agreement with a retrospective study of *T. brevifolia* that showed that 10-year increments were significantly larger following overstory removal than before the disturbance event (Bailey and Liegel, unpublished). These findings contradict the supposition that exposure to full sunlight causes reduced vigor or mortality of *Taxus* species (Anonymous 1992a; Crawford 1983). Increased needle pigmentation and mortality of some of the more exposed branches may occur following overstory removal (Anonymous 1992a; Crawford 1983; personal observation; Vance, unpublished data). Despite these changes, however, open-grown trees in this study tended to be more vigorous than shaded trees.

Morphology

It appears that *T. brevifolia* has sufficient phenotypic plasticity to adapt and even thrive under the drastic changes in microclimate that overstory removal entails. Branchiness (as indicated by the number of bifurcations and mass of branch samples) was positively associated with overstory openness. This increased branching resulted in a tendency for trees growing under shaded conditions to have relatively planar branches with herring bone type branching patterns, while open-grown trees had more multilayered branches and irregular branching patterns (personal observation). This pattern is in agreement with the response of other shade- tolerant species to overstory removal (Horn 1971; O'Connell and Kelty 1994; Steingraeber *et al.* 1979; Tucker *et al.* 1987; Veres and Picket 1982).

The negative relationship between specific leaf area and overstory openness is in keeping with that observed for *T. brevifolia* in another study in western Oregon (Vance and Kelsey 1992) and for other species (Dale and Causton 1992; Hughes 1965; Jones and McLeod 1990; Tucker *et al.* 1987). Under high light levels needles tend to be thicker while under low light photosynthetic surface is maximized (Beets and Lane 1987).

It was somewhat surprising that the ratio of foliage to total branch mass was not significantly related to overstory canopy for *T. brevifolia.* Veres and Pickett (1982) found that the foliage-to-branch ratio was significantly greater for shaded versus open grown *Lindera benzoin* shrubs. Open grown *T. brevifolia* trees have high rates of needle abscision, and foliage lifespan is shorter than on shaded

trees (personal observation). Foliage attrition may have been compensated for by increased foliage production on open grown trees, thus preserving the balance between stem and foliage mass.

Many shade-tolerant trees show limited plasticity in photosynthetic rates in response to increased light levels (Strauss-Debenedetti and Bazzaz 1991). However, this lack of plasticity can be compensated for by morphological changes that increase the quantity of photosynthetic tissues or establish a multilayered branch arrangement (O'Connell and Kelty 1994). Mitchell (1990) suggested that *T. brevifolia* has limited capability for increasing photosynthetic rates in response to increased light intensities. In this species an important mode of adaptation to increased light may be morphological changes to increase total surface area of photosynthetic tissue. Other changes include increase needle deflection (Crawford 1983; personal observation) and increased pigmentation. These changes may also allow avoidance of photoinhibition and damage from increased ultraviolet light.

The mechanisms of the response of growth and reproductive potential to overstory openness are not clear. Allocation to reproduction may increase in response to elevated light levels either due to increased carbon assimilation, through some hormonally mediated mechanism, or both. It is also possible that light is not the major factor determining strobilus production. Removal of the overstory drastically changes the microenvironment of the tree in many ways other than increased light levels. As Horn (1971, p. 15) states

> Any measurement of light on the forest floor, no matter how accurate and physiologically appropriate, confounds root and shoot effects since each bit of foliage overhead demands water and nutrients from below and sustains the activities of the roots that get them.

The most important effect of overstory removal may be a reduction in below ground competition, if this is the major limitation on growth and reproduction under intact canopy.

Nevertheless, increased light levels are certainly one of the more dramatic results of overstory removal, and there is ample evidence that shading limits flowering and seed production in other species (Dale and Causton 1992b; Devlin 1988; Niesenbaum 1993; Owens and Blake 1985; Schutte Dahlem and Boerner 1987; Silen 1973). Other factors such as SLA, which are known to be associated with light under controlled conditions, were also correlated with DIFN in this study. This suggests that DIFN was a reasonable index of the availability of PAR.

CONCLUSION

Growth and branch reproductive potential were significantly associated with overstory openness for these *T. brevifolia* populations. There were significant differences among the sites and between years both in the magnitude of reproductive potential and in the relationships with overstory openness. This suggests that factors associated with the different sites, such as climate, edaphic factors or topography, are important in determining reproductive potential. Factors associated with individual trees, such as microclimate or hereditary factors, may also play a role in determining reproductive potential.

The present study suggests that overstory removal is beneficial to *T. brevifolia* because it results in increased growth rates and reproductive potential. However, increased growth and reproductive potential do not necessarily indicate increased fitness, because these changes do not necessarily result in increased survival of offspring (Primack and Kang 1989). In fact, a related study (Chapter 4) shows that a number of factors such as pollination failure, predation by vertebrates, and spontaneous abortion combined to limit seed production at these sites, and there was no significant relationship between overstory openness and production of mature seeds. Therefore, while overstory removal does not necessarily harm *T. brevifolia* directly, nor does it necessarily result in increased fitness of the trees. Therefore, because overstory removal often results in substantial physical damage to understory trees, this may not represent a valid option for the management of *T. brevifolia* populations.

CHAPTER 4 FACTORS LIMITING SEED PRODUCTION OF *TAXUS BREVIFOLIA* IN WESTERN OREGON

INTRODUCTION

Seeds provide a wealth of important functions for plants, and seed production can play a crucial role in plant life history. Sexual reproduction is the major mode of introducing and maintaining genetic variation in plant populations. Seeds also provide an effective means of dispersal to favorable establishment sites. Food reserves contained in many seeds can aid in establishment and survival of germinants. Seeds serve as perennating organs as well, enduring conditions that would destroy adult plants (Harper 1977).

Evolutionary fitness largely depends on successful reproduction, and seed production is a major component of reproductive success. Therefore, ecological factors that affect heritable components of fecundity are potentially important sources of natural selection in plant populations (Primack and Kang 1989).

Plants commonly produce many more ovules than seeds (Stephenson 1981; Sutherland 1986; Sweet 1973), and the mechanisms and patterns of fruit and flower abortion can provide insight into the evolutionary significance of low seed-ovule ratios. The major factors responsible for ovule attrition are inadequate pollination (reviewed by Bierzychudek 1981; Burd 1994; Young and Young 1992), resource limitation (reviewed by Stephenson 1981; Willson and Burley 1983), genetic load (Charlesworth 1989; Sorensen 1969; Wiens *et al.* 1987), loss of seed to predators and pathogens (De Steven 1982; Janzen 1971; Louda 1982; Rauf *et*

al. 1985), and/or physical damage from abiotic factors such as frost (Owens and Blake 1985; Sweet 1973).

The relative importance of pollen limitation versus resource limitation has been a recurring theme in reproductive biology research. A common way of experimentally testing for pollen limitation is by adding supplemental pollen to plants growing under natural conditions and determining if there is an increase in seed production. However, several authors have pointed out shortcomings in this technique. Increased seed production might come at the expense of seed production in other portions of the same plant, or cause a reduction in future growth, reproduction, or survival of the manipulated plant (Bawa and Webb 1984; Janzen *et al.* 1980; Zimmerman and Pyke 1988). Indeed, it appears that in some plants pollen limitation can occur over the short term, but lifetime reproduction of a plant is primarily limited by resource availability (Ackerman 1989; Aker 1982; Zimmerman and Aide 1988). Other authors have pointed out that many plants are likely to be limited by both pollen and resources (Haig and Westoby 1988), and complex interactions between resource and pollen availability can occur. For example, plants can be limited by pollen when resources are abundant or limited by resources when pollen is abundant (Casper and Niesenbaum 1993). Alternatively, resources and pollen might affect different components of reproduction, as in a case where resources primarily affect ovule production and pollination primarily affects the proportion of ovules that develop into seed (Campbell and Halama 1993).

For many plants the relative importance of pollen or

resource limitation of reproduction is likely to depend on resource levels. One source of variation in resource availability for understory plants is the density of the overstory canopy. Seed production of many understory plants is limited by low light availability (Bunnell 1990; Dale and Causton 1992; Devlin 1988; Lee 1989; Niesenbaum 1993), and successful sexual reproduction may only occur in elevated light levels found beneath overstory gaps (Thompson and Willson 1978).

Predispersal seed predation is another factor that can interact with pollen and resource availability in limiting seed production. For example, under high resource availability predation might limit seed production. Alternatively if resources and predation were low, resources might limit seed production (Ehrlén 1992; Louda 1982). Therefore, it is necessary to take predispersal seed predation into account when considering the importance of resource and pollen limitation of seed production.

The purpose of the present study is to determine the importance of pollen limitation, resource limitation and predation in limiting seed production of the understory tree Pacific yew (*Taxus brevifolia* Nutt., Taxaceae). Pollen limitation is assessed through pollen exclusion and pollen supplementation experiments. The relationship between ovule development and pollen availability is also explored. Resource limitation is assessed in two ways. The relationship between overstory openness and seed production is examined. A positive relationship would indicate resource limitation of seed production, because overstory openness provides an index of resource availability (Canham

and Marks 1985). Overstory openness was previously shown to be positively associated with branch reproductive potential (strobilus production) for the trees examined in this study (Chapter 3). The relationships between growth and reproduction, and between current and previous rates of seed production are also examined, because negative relationships would suggest resource limitation of seed production (Harper 1977; Sweet 1973). Finally, predispersal seed predation is indexed through a predator exclusion experiment. Variability in the importance of pollen, resources and predators is assessed for four different study sites over two years.

METHODS

Study Organism

T. brevifolia occurs from northern California as far north as Southeast Alaska, and inland to western Montana (Bolsinger and Jaramillo 1990). It is highly shade-tolerant and is found most frequently in the understory of late seral forests, although it is not an obligate understory species (Busing *et al.* 1995; Chapter 3; McCune and Allen 1985).

T. brevifolia is dioecious and wind-pollinated. Mature female strobili consist of a single seed with a hard integument, surrounded by a bright red cuplike aril. In western Oregon, pollination occurs in March and April and seed maturation begins in late July and continues through October (Chapter 2). Seeds are consumed and dispersed by a variety of birds and rodents, including jays (*Perisoreus canadensis* and *Cyanocitta stelleri*), nuthatches (*Sitta canadensis*), chickadees (*Parus atricapillus*) and chipmunks

(*Tamias townsendii*) (Chapter 1; Nomenclature follows Peterson 1990).

Study Design and Tree Selection

This study was conducted on 4 sites in western Oregon (Table 2.1). The main criteria for site selection were the presence of at least 10 sexually mature *T. brevifolia* trees per hectare and a range of canopy cover above the *T. brevifolia* trees. Working systematically from an arbitrary starting point, all *T. brevifolia* trees greater than 3 m tall and 5 cm basal diameter were mapped until at least 30 male and 30 female trees were included. Trees separated by 2 m or less were considered ramets of the same genet, and were mapped as one tree. None of the trees selected for intensive study were within 3 m of one another.

In order to ensure an adequate range of overstory canopy cover, trees were selected by stratified random sampling. Trees were subjectively assigned to two groups: those growing under open canopy (OPN) and those under canopy cover (CAN). 7 male and 7 female trees were randomly selected from each canopy grouping, for a total of 28 trees per site. This selection technique resulted in two spatially separated populations per site, as described below. For convenience, 'site' will refer to an entire drainage and 'plot' will refer to a canopy grouping within a site.

Study Sites

The Higher Cascade 1 site (HC1) was located in the Snow Creek drainage of the Willamette National Forest (44° 23' 30" N; 122° 15' W). Overstory trees in the OPN plot were

harvested in a 1990 clearcut. The CAN plot was located approximately 1 km upslope from the OPN plot.

The Higher Cascade 2 site (HC2) was located in the Hackleman Creek drainage of the Willamette National Forest (44° 24' N; 122° 02' W). Overstory trees for the OPN plot were harvested in a 1978 salvage logging operation, and substantial windthrow of remaining trees had occurred since that time. The CAN plot was located approximately 1.5 km downslope from the OPN plot.

The Low Cascade site (LC) was located in the McCrae Creek drainage of the H.J. Andrews Experimental Forest (44° 15' N; 122° 11' W). Canopy gaps in the OPN plot were created by a 1981 clearcut and construction of a road in the 1950's. The CAN plot was contiguous with the OPN plot.

The Willamette Valley site (VAL) was located in the west fork of the Oak Creek drainage of the McDonald Research Forest in the foothills of the Oregon Coast Range (44° 35' N; 123° 35' W). Overstory trees in the OPN plot were harvested in a 1984 clearcut. The CAN plot was located approximately 2 km downslope from the OPN plot.

More detailed information about study sites is provided in Chapter 2.

Ovule and Seed Production

Ovule production was determined on subsampled branches, and no attempt was made to estimate the productivity of entire trees. This method should reveal trends in the data that might otherwise have been obscured by errors in estimates of whole tree productivity. There was no evidence of a relationship between tree size and ovule and seed

production per branch unit, or between tree size and overstory canopy (Chapter 3).

In 1993 8 branch tips were selected on each tree by stratified random sampling. Each tree crown was first divided into upper and lower sectors and 4 branches were randomly selected from each sector. Beginning at the basipetal portion of the branch, successive bifurcations were randomly chosen until arriving at a branch apex. Branches were measured 30 cm basipetal from the selected apex and marked with nylon flagging. All branch tips were sampled acropetal to the 30 cm mark. In May, June and July of 1993 the number of ovules (approximately the same as number of strobili, as *T. brevifolia* is primarily uniovulate) was counted and categorized as developing, nondeveloping or mature, based on external morphology. Developing ovules were green and expanding, while nondeveloping ovules were unexpanded and yellow.

From August until October of 1993 the number of mature seeds per branch was counted at two-week intervals. Seeds with red arils covering the entire seed coat were considered mature. Estimates of seed production included the total number of mature seeds observed, the total number of empty receptacles (indicative of removal of mature seeds) and the number of healthy, developing seeds present at the final census. Mature seeds and empty receptacles were removed at each census in order to avoid duplication in counting.

Ovule and seed production in 1994 was estimated in much the same way as in 1993, except trees were visited once per month rather than every 2 weeks. In May of 1994 30 cm was remeasured on each selected branch tip and new branches were

selected to compensate for mortality (≈1% of all branches). In late September of 1994 all branches were clipped at the 1994 flagging and transported to the lab. All growth distal to 1994 bud scars was clipped and separated. All branch material was dried at 65° C for 48 hours and stems, foliage and 1994 growth were weighed separately.

The development and production of ovules and seeds were assessed at several stages, and specific terms were defined to facilitate presentation of results (Table 4.1).

Table 4.1 Terms defined to describe development and production of seeds and ovules.

Ovule Production (OVPROD)- Maximum number of developing and nondeveloping ovules observed in May, June and July censuses.
Ovule Development (OVDV)- Number of green and expanded ovules observed in July.
Ovule Development Efficiency (OVDVEF)- Ovule Development/Ovule Production.
Seed Production (SDPROD)- Number of mature seeds, receptacles and developing seeds observed at final census.
Seed Development Efficiency (SDDVEF)- Seed Production/Ovule Development.
Seed Efficiency (SDEF)- Seed Production/Ovule Production.

Vertebrate Exclusion

Five trees per site were selected in 1993 for a vertebrate exclusion experiment. Four branches per tree were randomly selected and covered in July with bags made from one mm nylon mesh. Seed production was determined in late September.

In 1994 half the branches were bagged on all trees that had developing seeds as of July (excluding trees receiving

supplemental pollination, see below). On trees that had been bagged in 1993, two branches were randomly selected from those that had been bagged previously and two branches were selected that had not been bagged in 1993. This was done to randomize carryover effects from bagging in the previous season.

Pollination

Pollen Exclusion

Pollen was completely excluded from thirty-eight branch tips on thirteen trees in 1993 and 1994 in order to determine the rate of seed development in the absence of pollination. Before pollen shedding was observed at each site, branch tips were covered with paper pollen-exclusion bags. Cotton batting was wrapped around the branch and the bags were secured over the cotton with twist ties.

Pollen Supplementation

A pollen supplementation experiment was performed at the HC1 and VAL sites in 1994. The HC1 and VAL sites were chosen because considerable attrition of ovules was observed on these sites following pollination in 1993, possibly indicating pollen limitation.

In 1994 five female trees were randomly selected on each site from all trees that produced at least two strobili per 30 cm branch in 1994. Twenty-one 30-cm branch segments were selected as described for ovule and seed production censuses. One branch per tree was designated to be covered with a pollen exclusion bag and receive hand-pollination ('exclusion and pollination' treatment), ten open-pollinated

branches were randomly chosen to receive hand-pollination ('supplemental pollination' treatment) and ten open-pollinated branches received no supplemental pollen (control).

Pollen was collected by clipping branches from several male trees per site just before pollen began shedding. Cut ends were placed in water at room temperature overnight and pollen was collected on waxed paper. Pollen was stored in corked glass vials at 4°C to be used within one week of collection. Pollen viability was assessed by placing hydrated pollen in a nutrient and electrolyte solution (Brewbaker and Kwack 1963) overnight and examining grains at 250X. Grains with intact cytoplasm which shed exines and elongated were designated as viable. Pollen used in hand-pollinations had greater than 90% viability.

Hand-pollinations were performed in late March and April, at the time of peak receptivity of female trees. Criteria for receptivity were the emergence of the ovule from beneath the bud scales and the presence of a pollination drop at the micropyle of the ovule (Chapter 1). Receptivity was protracted at the VAL site, and there was much variability among trees, while receptivity was more synchronized at the HC1 site (Chapter 2). Therefore, hand-pollinations were performed on five dates at VAL and one at HC1.

At the time of hand-pollination all 'control' branches were first covered with pollen exclusion bags and 'exclusion and pollination' branches were uncovered. A soft paintbrush was used to apply pollen directly to the micropyles of all visible ovules on 'exclusion and

pollination' and 'supplemental pollination' branches. Bags were then removed from 'control' branches and 'bagged and pollinated' branches were rebagged.

The effect of supplemental pollination was assessed by comparing Ovule Development Efficiency and Seed Efficiency between 'supplemental pollination' and 'control' branches (Table 4.1).

Pollen Availability

Pollen production was indexed for each plot using the method described for female strobilus production, except 20 cm branch segments were used rather than 30 cm segments (Chapter 3). Crown volume was estimated for all male trees within a 30 m radius of each female study tree. Crown measurements consisted of height (h) and average crown width (r), and volume was estimated by assuming a conical crown shape ($(1/3)\cdot\pi r^2\cdot h$).

Increment Coring

To determine ages and growth rates of the study trees, an increment core was extracted 30 cm above the base on the north face of each tree. Cores were analyzed under a stereo microscope (30x) using a razor blade to expose a clean, smooth surface from which increments could be easily discerned. The total number of increments were counted and the width of the first five full increments proximal from the cambium was measured. In some cases age had to be estimated because some increments were obliterated by heartwood rot.

Overstory Canopy

Overstory canopy openness above and to the south of each study tree was estimated using the model LAI-2000 plant canopy analyzer (LI-COR, Lincoln, NE, USA). This instrument derives estimates of canopy cover from measurements of diffuse radiation. A refernce sensor placed in a clearing automatically logged light readings every 30 s while readings were taken above each *T. brevifolia* tree with a measurement sensor. Both sensors were levelled and pointed in exactly the same direction. Because of the difficulty in finding sufficiently large open areas near the *T. brevifolia* populations, a 270° lens cap was used on both sensors. Measurements were taken in a southwesterly direction between 7 am and 11 am and in a southeasterly direction between 4 pm and 8 pm, because direct sunlight confounds readings. All measurements were taken under relatively uniform sky conditions, usually on cloudless days. Most measurements were taken during July and August of 1993.

The value used in this study was Diffuse Noninterceptance (DIFN), an estimate of the amount of sky visible from beneath the canopy. DIFN ranges from 0 (completely closed canopy) to 1 (no canopy) (Welles and Norman 1991). The average of the southwest and southeast DIFN readings is an estimate of the openness of the canopy directly above and surrounding the southern aspect of each *T. brevifolia* tree. The southern aspect was measured on the assumption that this was the location of the most important canopy gaps at this latitude (i.e. those that resulted in the most significant increases in understory light levels).

Analysis

Relationships among continuous variables such as DIFN, Ovule Development, Seed Efficiency, growth, and treatment effects were assessed using the multiple linear regression procedure of SAS (SAS Institute 1987). Variables were transformed where appropriate to correct for heteroscedasticity. Separate slopes and intercepts were fitted for each site, and regression lines were compared among sites by systematically combining indicator variables and assessing the reduction in the regression sum of squares relative to the number of variables removed from the model (Neter and Wasserman 1974). Final models were the most parsimonious models that yielded an F-statistic greater than expected at the 95% confidence level when compared with the next simplest model.

Paired t-Tests were used for comparison of treatment effects with trees as a blocking factor (e.g. comparing bagged to unbagged branches within a year or seed production between years). For unpaired comparisons, (e.g. testing for the effect of pollen addition within an individual tree, or comparing treated to untreated trees), unpaired t-Tests were used. For cases where transformations failed to correct for heteroscedasticity, the Wilcoxon Signed-Rank Test was used for paired comparisons and the Wilcoxon Rank-Sum Test was used for unpaired comparisons (Mosteller and Rourke 1973).

RESULTS

Patterns of Attrition

Two distinct phases of attrition were observed (i) an early phase shortly after the time of pollination in which

nondeveloping ovules were unexpanded and yellow, and (ii) a late phase from late July through October in which aborted seeds were nearly full size but misshapen and hollow, and immature developing seeds disappeared from the branches. Over the 2 years of this study, site averages for Ovule Development Efficiency (which reflects early phase attrition) ranged from 0.09 to 0.71 and site averages for Seed Efficiency (which reflects all attrition) ranged from 0.05 to 0.34 (Table 4.2).

Ovule Development Efficiency was highest at HC2, followed by HC1, LC and VAL, in that order. The order was nearly the same for Seed Efficiency, which was highest at HC2, intermediate at HC1 and LC and lowest at VAL in both years of the study (Table 4.2).

Levels of attrition also varied somewhat between years. Ovule development efficiency was significantly higher in 1994 than in 1993 for sites LC and VAL, higher in 1993 at site HC2 and not significantly different between years at site LC. Seed efficiency was significantly different between years only at site LC.

Pollen Limitation

Pollen Exclusion

Of a total of 380 ovules on branches bagged to exclude pollen, only 2 ovules located on the same branch were developing (green and expanded) in July. These 2 ovules developed into apparently healthy, filled seeds. Although there were no signs that the pollen exclusion bag had been compromised, the possibility of pollen contamination cannot

Table 4.2 Mean ovule development and seed efficiencies by year and site. OVDVEF- Ovule Development Efficiency: mean Ovule Development/ovule production per 30 cm branch segment; SDEF- Seed Efficiency: mean Seed Production/Ovule Production per 30 cm branch segment. More detailed explanations are provided in Table 4.1 and methods. N- number of trees. Letters indicate homogeneous groups within years as determined by Duncan's Multiple Range Test (SAS Institute 1987). Values for "*P*, 1994=1993" are probablilities from Wilcoxon Signed-Rank Test for differences between years in tree means.

	1993				1994				*P*, 1994=1993			
	HC1	HC2	LC	VAL	HC1	HC2	LC	VAL	HC1	HC2	LC	VAL
N	14	16	14	14	14	15	14	15	14	15	14	14
OVDVEF	0.51^{b}	0.76^{a}	0.20^{c}	0.09^{d}	0.59^{b}	0.71^{a}	0.45^{c}	0.17^{d}	0.28	0.01	<0.001	0.03
SDEF	0.15^{ab}	0.24^{a}	0.11^{b}	0.05^{c}	0.20^{b}	0.34^{a}	0.21^{b}	0.05^{c}	0.35	0.15	0.001	0.90

be ruled out. There have been reports of low levels of apomixis in the genus *Taxus* (Allison 1993; Orr-Ewing 1957), and this possibility warrants further investigation. However, if apomixis does occur in *T. brevifolia*, it appears to be uncommon, suggesting that pollen is usually necessary for ovule development to occur.

Pollen Supplementation

Hand-pollination on bagged branches resulted in Ovule Development Efficiency ranging from 0.6 to 0.9 (mean=0.7) at VAL and 0.5 to 0.8 at HC1 (mean=0.7). Therefore the the hand-pollination technique was effective in stimulating ovule development.

Ovule Development Efficiency (OVDVEF) was considerably less than 1 on all trees for all treatments (Figure 4.1A) and Seed Efficiency was less than 0.1 over all treatments on all trees at HC1, and ranged as high as 0.33 at VAL for hand-pollinated branches (Figure 4.1B). Ovule Development Efficiencies were significantly higher on branches receiving supplemental pollination than on control (open-pollinated) branches on 4 of 5 trees at the VAL site and 2 of 5 trees at the HC1 site (Figure 4.1A). Seed Efficiency (SDEF) was improved by pollen supplementation on 2 of 5 trees at VAL and it was not significantly improved on any trees at HC1 (Figure 4.1B). On the whole tree level, Ovule Development Efficiency was significantly greater with supplemental pollination at the VAL site but not at the HC1 site, while Seed Efficiency was significantly improved at both sites (Table 4.3).

Table 4.3 Effect of supplemental hand-pollination on Ovule Development and Seed Efficiency in 1994. Ovule Development Efficiency- the proportion of ovules that were green and expanded in July; Seed Efficiency- Seed Production/Ovule Production (see table 4.1 for further definition); Supplemented- branches receiving supplemental hand-pollination; Control- open-pollinated branches.

Variable	Site	Supplemented	Control	p*
Ovule Development Efficiency	HC1	0.72	0.64	0.14
	VAL	0.58	0.19	0.007
Seed Efficiency	HC1	0.06	0.03	0.008
	VAL	0.15	0.06	0.02

* Significance as determined by paired t-Test. Results were qualitatively the same with Wilcoxon Signed-Rank Test. N=5 for both sites.

There was no evidence that increases in Ovule Development Efficiency or Seed Efficiency on pollen-supplemented branches occurred at the expense of ovule or seed development on control branches. Seed Efficiency on control branches in the year of pollen-supplementation was not significantly different from Seed Efficiency on the same trees in the previous year (mean difference=0.049, paired $t=1.17$, $p=0.27$). Similarly, Seed Efficiency on control branches on trees receiving supplemental pollination was not significantly different from Seed Efficiency on unmanipulated trees at the same site in the same year (Table 4.4).

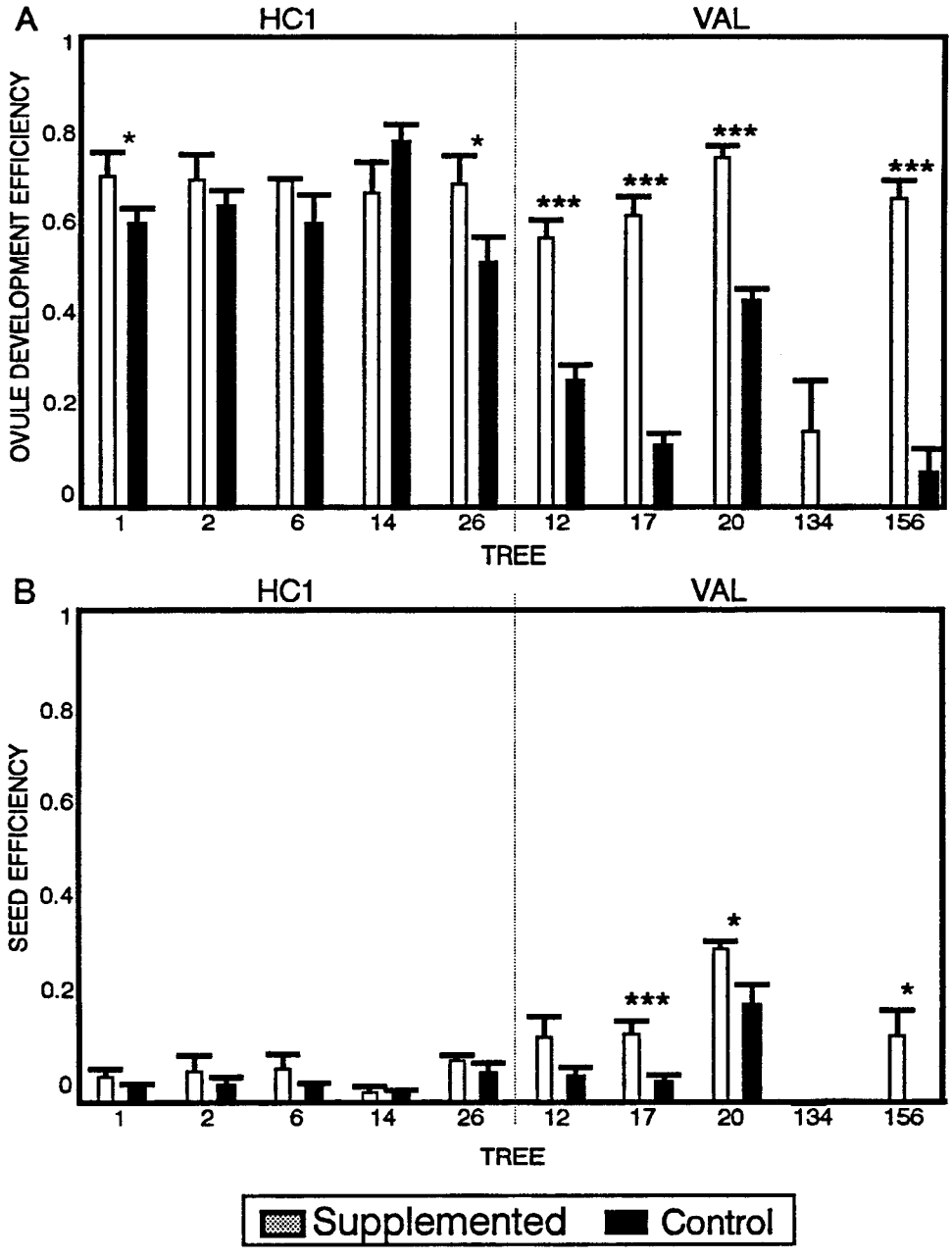

Figure 4.1 Effect of supplemental hand pollination within trees on sites HC1 and VAL in 1994. **A.** Ovule Development Efficiency= Seed Production/Ovule Development per 30 cm branch segment; **B.** Seed Efficiency= Seed Production/Ovule Development per 30 cm branch segment. Supplemented- open-pollinated branches receiving supplemental hand-pollination; Control- open pollinated branches. Comparisons between supplemented and control were made by t-Tests of branch means. Results were qualitatively the same with arcsine square root transformed data and the Wilcoxon Rank Sum Test. Error bars represent 1 standard error. *- $p<0.05$; ***- $p<0.001$.

Table 4.4 Comparison of average Seed Efficiency on control branches of pollen-supplemented trees with Seed Efficiency on untreated trees. Supplemented- trees receiving supplemental hand-pollination on some branches; Unsupplemented- trees not used in pollen supplementation experiment; N- number of trees in treatment group; Seed Efficiency- average Seed Efficiency of open-pollinated branches (see table 4.1 for further definition); *P*- probability that means are not significantly different, as determined by t-Test.

Site	Treatment	N	Seed Efficiency	*P*
HC1	Supplemented	5	0.084	0.64
	Unsupplemented	9	0.067	
VAL	Supplemented	5	0.11	0.27
	Unsupplemented	9	0.035	

Pollen Availability

In 1994 there was a significant relationship between pollen production and density of male trees versus Ovule Development Efficiency (Table 4.5). The slope was positive for pollen production and was not significantly different for the four sites. The slope for male density was positive and significant for HC2 and LC but not significant for HC1 and VAL. In addition, there was a significant negative slope for the square of male density, which was not significantly different for the four sites. This indicates a nonlinear relationship for male density. The results were quite different for 1993 Ovule Development Efficiency. Male density did not account for significant variation in Ovule Development Efficiency, and pollen production was only significant at site LC (Table 4.5).

The intercepts of the lines were significantly greater than 0 for 4 sites in 1994 and 3 sites in 1993. The non-

Table 4.5 Regression equations for Ovule Development Efficiency versus pollen availability. OVDVEF- Ovule Development Efficiency: mean Ovule Development/Ovule Production per 30 cm branch (Table 4.1); POL- mean male strobilus production per 20 cm branch segment in vicinity of female trees; MLDEN- estimate of total canopy volume for male trees within 30 m of female trees; X_i- indicator variable for site i; 1=High Cascade 1, 2=High Cascade 2, 3=Low Cascade, and 4=Willamette Valley. If more than one subscript appears then the parameter estimate was not significantly different for those sites. df- total degrees of freedom; R^2- coefficient of determination. Standard errors for parameter estimates are in parentheses below each estimate. ns- not significantly different from 0 at $p \leq 0.05$.

Model		df	R^2
ARCSIN(SQRT(OVDVEF93))	$= \underset{(0.05)}{0.76 \cdot X_1} + \underset{(0.04)}{1.07 \cdot X_2} + \underset{(0.04)}{0.31 \cdot X_{34}} + \underset{(0.0008)^{ns}}{0.00085 \cdot POL \cdot X_{124}} + \underset{(0.003)}{0.010 \cdot POL \cdot X_3}$	50	0.85
ARCSIN(SQRT(OVDVEF94))	$= \underset{(0.06)}{0.82 \cdot X_1} + \underset{(0.09)}{0.67 \cdot X_2} + \underset{(0.08)}{0.43 \cdot X_3} + \underset{(0.06)}{0.30 \cdot X_4} + \underset{(0.04)^{ns}}{0.03 \cdot MLDEN \cdot X_{14}} + \underset{(0.05)}{0.19 \cdot MLDEN \cdot X_{23}} - \underset{(0.005)}{0.02 \cdot MLDEN^2} + \underset{(0.0009)}{0.0035 \cdot POL}$	50	0.72

zero intercepts suggest that pollen travelled further than 30 m in these plots.

Overstory

Overstory openness (DIFN) was positively associated with the number of developing ovules at all sites in 1993 and at 3 of 4 sites in 1994 (Table 4.6; Figure 4.2A,B;). In 1993 the relationship between DIFN and Ovule Development was not significantly different for the 4 sites, although the intercepts were higher for the two higher Cascade sites than for LC and VAL (Table 4.6; Figure 4.2A). Overstory openness accounted for 68% of the observed variation in Ovule Development (Table 4.6).

In 1994 sites HC2, LC and VAL had the same positive relationship between DIFN and Ovule Development, although the intercepts differed (Table 4.6; Figure 4.2B). There was no relationship between overstory openness and Ovule Development at site HC1 in 1994 (Table 4.6). The fit of the regression equation was also lower than in 1993 (R^2=0.45, Table 4.6).

In contrast to Ovule Development, no significant association was detected between DIFN and Seed Efficiency in 1993 and 1994 (Table 4.6; Figure 4.2C,D). This lack of a significant relationship was due to higher attrition of developing seeds under open-canopy conditions. This is illustrated by the negative relationship between overstory openness and Seed Efficiency for HC1, HC2 and LC in 1993 and all 4 sites in 1994 (Table 4.6; Figure 4.2 E,F).

Table 4.6 Regression equations for Ovule Development, Seed Production and Seed Efficiency versus overstory openness (DIFN) on unbagged branches. OVDV- mean number of ovules develping in July per 30 cm branch segment; SDPROD- mean Seed Production per 30 cm branch segment; SDEF- mean Seed Production/Ovule Production per 30 cm branch segment. Standard errors for parameter estimates are in parentheses below each estimate. Other abbreviations are as described for Table 4.5.

Model	df	R^2
1993		
$LN(OVDV) = \underset{(0.17)}{2.10 \cdot X_{12}} + \underset{(0.19)^{ns}}{0.27 \cdot X_{34}} + \underset{(0.29)}{1.12 \cdot DIFN}$	50	0.68
$LN(SDPROD) = \underset{(0.16)}{1.13 \cdot X_{12}} + \underset{(0.17)^{ns}}{0.33 \cdot X_{34}} + \underset{(0.27)^{ns}}{0.48 \cdot DIFN}$	50	0.30
$ARCSIN(SQRT(SDEF)) = \underset{(0.05)}{0.53 \cdot X_{12}} + \underset{(0.06)}{0.31 \cdot X_3} - \underset{(0.12)^{ns}}{0.09 \cdot X_4} - \underset{(0.10)}{0.25 \cdot DIFN \cdot X_{123}} + \underset{(0.18)}{0.49 \cdot DIFN \cdot X_4}$	50	0.17
1994		
$LN(OVDV) = \underset{(0.37)}{2.10 \cdot X_1} + \underset{(0.24)}{1.49 \cdot X_2} + \underset{(0.26)^{ns}}{0.48 \cdot X_3} - \underset{(0.37)^{ns}}{0.29 \cdot X_4} + \underset{(0.57)^{ns}}{0.23 \cdot DIFN \cdot X_1} + \underset{(0.48)}{2.41 \cdot DIFN \cdot X_{234}}$	50	0.45
$LN(SDPROD) = \underset{(0.12)}{0.73} + \underset{(0.22)^{ns}}{0.16 \cdot DIFN}$	50	0.01
$ARCSIN(SQRT(SDEF)) = \underset{(0.05)}{0.46 \cdot X_{123}} + \underset{(0.10)^{ns}}{0.32 \cdot X_4} - \underset{(0.09)}{0.18 \cdot DIFN}$	50	0.17

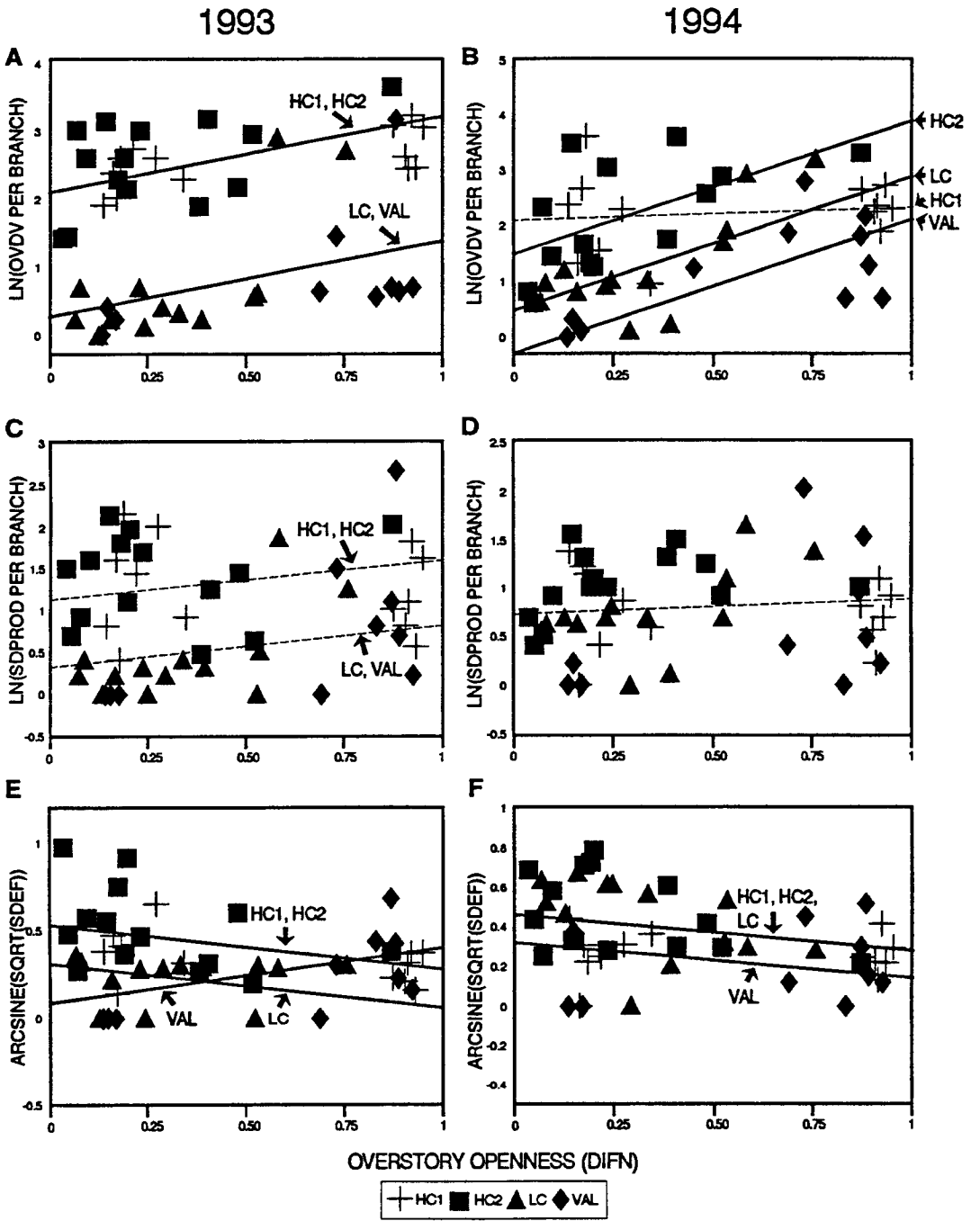

Figure 4.2 Regressions of Ovule Development (OVDV), Seed Production (SDPROD) and Seed Efficiency (SDEF) versus overstory openness (DIFN). Each point represents mean values for individual trees. Symbols represent sites. Broken lines have slopes that are not significantly different from o. Lines correspond to equations in Table 4.6. Terms are defined in Table 4.1.

Predation

Branches bagged to exclude vertebrates had significantly higher mean Seed Development Efficiencies than unbagged branches at the HC1, HC2 and LC sites in 1993 and 1994 (Figure 4.3). Mean Seed Development Efficiencies were also higher for bagged branches than for unbagged branches at VAL, but the difference was not significant.

Most of the difference in Seed Development Efficiency between bagged and unbagged branches appeared to be due to predation. The main predator of immature seed observed in this study was Townsend's chipmunk (*Tamias townsendii*). *T. townsendii* was common throughout the study area and was observed to destroy immature seeds at all sites. The mode of foraging involved hanging from the underside of branches and systematically removing developing seeds, biting a hole in the poisonous seed integument and removing the seed contents. Large quantities of seed integuments with bite holes were found beneath all productive trees in this study (personal observation).

Removal and dispersal of mature seed might also have accounted for some of the difference in Seed Development Efficiency between bagged and unbagged branches. However, errors in estimates of Seed Production were minimized by censusing Seed Production every two weeks in 1993. Also, the inclusion of empty receptacles in estimates of Seed Production compensated for the removal of mature seed between censuses. Spontaneous abscission of the strobilus at the base of the receptacle was relatively rare: 82% of 754 seeds produced on bagged branches in 1993 were still attached to the branches in October.

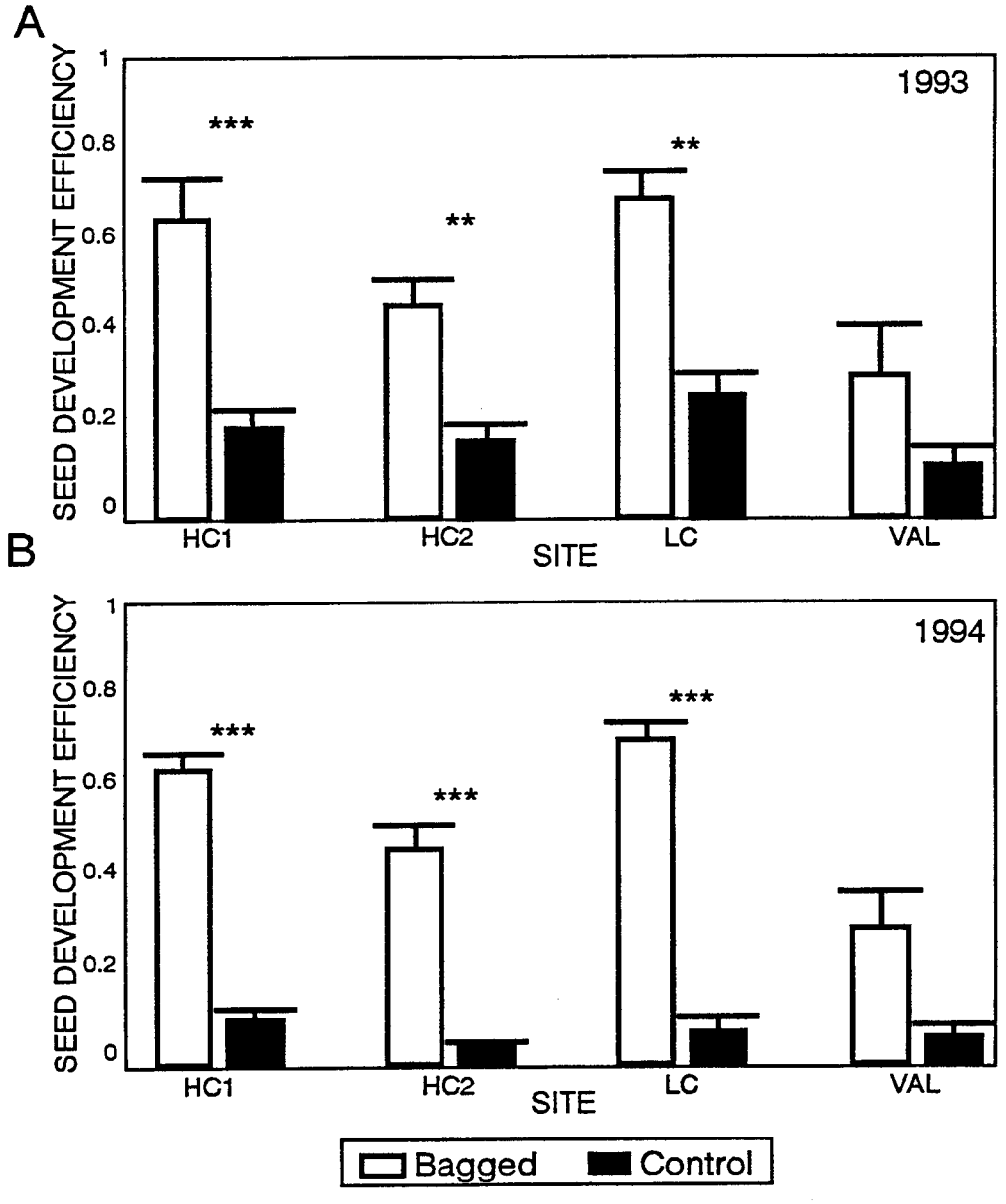

Figure 4.3 The effect of bagging to exclude vertebrates on Seed Development Efficiency. **A.** 1993; **B.** 1994. Comparisons made by paired t-Test of tree means. Results were qualititatively the same with arcsine square root transformed data and the Wilcoxon Signed-Rank Test. Error bars represent 1 standard error. **-p<0.01; ***-p<0.001.

Seed Production was censused monthly rather than biweekly in 1994, so underestimates of Seed Production on unbagged branches were more likely than in 1993. Despite this difference, however, the bagging effect in 1993 was not significantly different from that in 1994 (Wilcoxon Signed Rank Test, N=19, W=0.63, p=0.82).

In contrast to unbagged branches, average Seed Production on branches bagged to exclude predators was positively associated with DIFN at sites HC1, HC2 and LC in 1993 and 1994, although the fit of the equations was not as good as for Ovule Development (Table 4.7; Figure 4.4C). The analysis was not performed for trees bagged at the VAL site because no seed was produced by trees growing under overstory cover, and the range of DIFN above bagged trees was inadequate. Average Seed Efficiency was not associated with DIFN in 1993 (Table 4.7; Figure 4.4E). 1994 Seed Efficiency was significantly and positively associated with DIFN (Table 4.7; Figure 4.4F). The fit for 1994 Seed Production and Seed Efficiency was better than for 1993, perhaps because more trees were included in the analysis in 1994.

The difference between Seed Development Efficiency on bagged branches and Seed Development Efficiency on unbagged branches was also used as a response variable in regressions versus overstory openness as a measure of the effectiveness of bagging. Because Seed Efficiency was censused twice as frequently on unbagged branches in 1993 as in 1994, two values were used for the bagging effect in 1993, one based on cumulative Seed Efficiency and one based on the number of seeds present on the branches at the final census only. The

Table 4.7 Regression equations for Ovule Development, Seed Production and Seed Efficiency versus overstory openness (DIFN) on branches bagged in July to exclude predators. Analyses were performed on a subset of trees used in unbagged analyses, but with different branches. Trees from the VAL site were not included in these analyses due to an inadequate range of overstory openness. Standard errors for parameter estimates are in parentheses below each estimate. Abbreviations are as described in Tables 4.5 and 4.6.

Model	df	R^2
1993		
$LN(OVDV) = \underset{(0.28)}{2.08 \cdot X_1} + \underset{(0.20)}{2.54 \cdot X_2} + \underset{(0.40)^{ns}}{0.58 \cdot X_3} + \underset{(0.44)}{0.96 \cdot DIFN \cdot X_{12}} + \underset{(0.80)}{3.01 \cdot DIFN \cdot X_3}$	20	0.59
$LN(SDPROD) = \underset{(0.33)}{1.34 \cdot X_{13}} + \underset{(0.29)}{2.10 \cdot X_2} + \underset{(0.57)}{1.19 \cdot DIFN}$	20	0.21
$ARCSIN(SQRT(SDEF)) = \underset{(0.12)}{0.56 \cdot X_{13}} + \underset{(0.10)}{0.85 \cdot X_2} + \underset{(0.21)^{ns}}{0.08 \cdot DIFN}$	20	0.18
1994		
$LN(OVDV) = \underset{(0.19)}{1.62} + \underset{(0.42)}{1.31 \cdot DIFN}$	30	0.23
$LN(SDPROD) = \underset{(0.19)}{1.48 \cdot X_{12}} + \underset{(0.26)}{0.83 \cdot X_3} + \underset{(0.38)}{1.63 \cdot DIFN}$	30	0.46
$ARCSIN(SQRT(SDEF)) = \underset{(0.07)}{0.54 \cdot X_{13}} + \underset{(0.06)}{0.84 \cdot X_2} + \underset{(0.12)}{0.36 \cdot DIFN}$	30	0.42

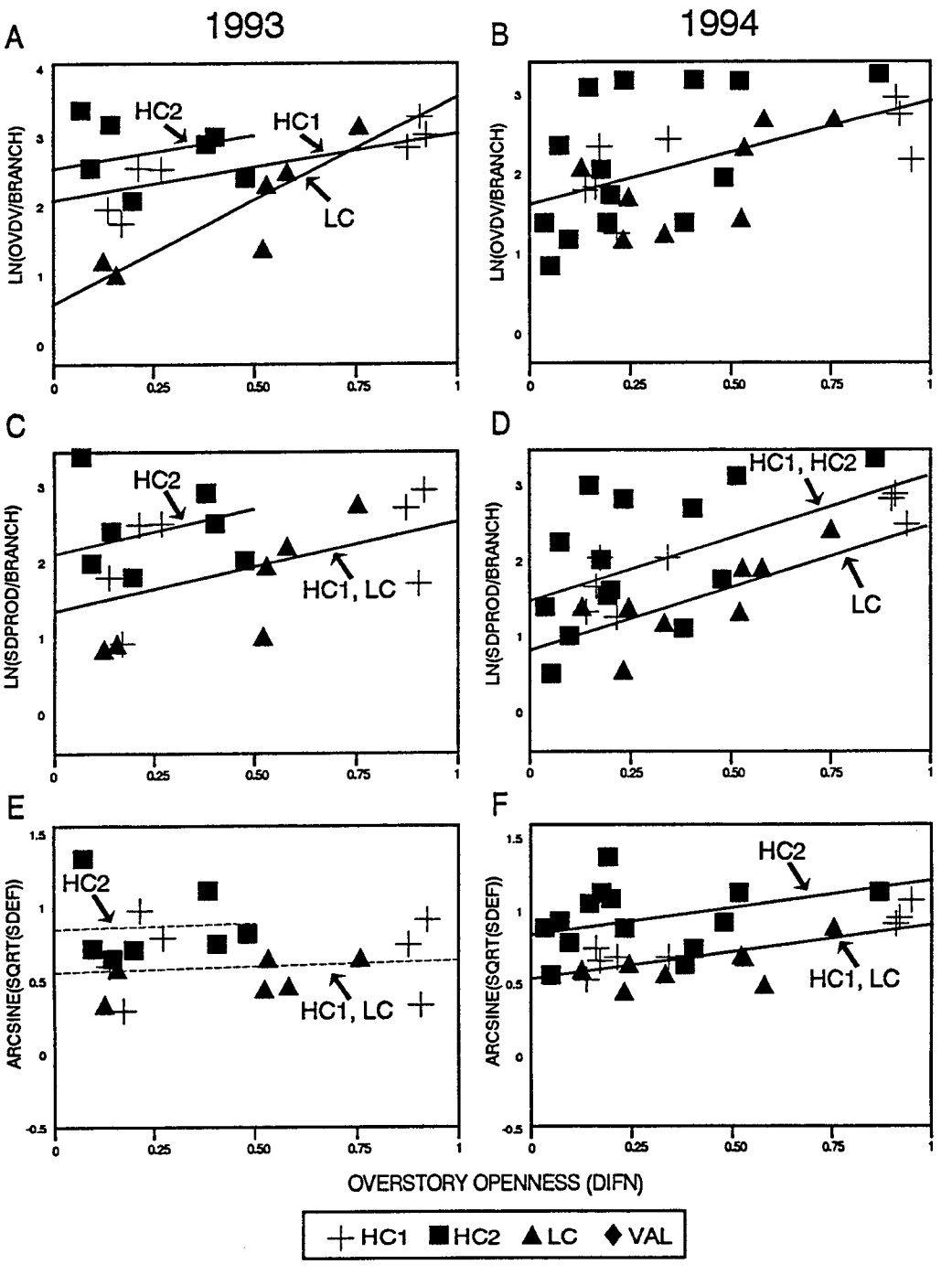

Figure 4.4 Regressions of Ovule Development (OVDV), Seed Production (SDPROD) and Seed Efficiency (SDEF) versus overstory openness (DIFN) for branches bagged to exclude vertebrates. Each point represents average values for individual trees. Symbols represent sites. Broken lines have slopes that are not significantly different from 0. Lines correspond to equations in Table 4.7.

former value is the best estimate of the effect of predators on Seed Development Efficiency, while the latter value is most comparable to the 1994 bagging effect.

The effect of bagging was not significantly associated with DIFN at any of the sites in 1993, regardless of which measure of Seed Efficiency on unbagged branches was used (Table 4.8; Figure 4.5A,B). The effect of bagging was positively associated with DIFN in 1994 at all sites (Table 4.8; Figure 4.5C).

Resource Limitation

1994 Ovule Production, Seed Development Efficiency and growth of branches that had been bagged in 1993 were compared to branches not bagged in 1993. There was no significant difference in any of these values between groups, so no carryover of bagging effects between years was detected (Table 4.9). 1994 Ovule Production on bagged branches was not significantly associated with Seed Development Efficiency in 1993. 1994 Seed Development Efficiency was positively associated with 1993 Seed Development Efficiency (Table 4.10). Thus there was no evidence for tradeoffs between prior Seed Development Efficiency and current reproductive output.

Regressions of the proportion of developing ovules that later aborted on bagged branches versus 5-year increment growth and 1994 growth revealed no strong relationships (Table 4.10). The only slope value that was significant was the proportion of undeveloped ovules in 1993 versus 1994 branch growth at VAL. However, this could have been a spurious correlation because only three trees were included

Table 4.8 Equations for regressions of the effect of bagging versus overstory openness (DIFN). DIFFCUM93- difference between bagged and unbagged branches in Seed Development Efficiency (Seed Production/Ovule Development per 30 cm branch segment), based on cumulutive Seed Production in 1993 (totals of bimonthly censuses); DIFF93- difference between bagged and unbagged branches in Seed Development Efficiency, based on a single census in September 1993; DIFF94- difference in Seed Development Efficiency between bagged and unbagged branches, based on a single census in September 1994. Standard errors for parameter estimates are in parentheses below each estimate. Other abbreviations are as described for Table 4.7.

Model	df	R^2
$\text{ARCSIN(SQRT(DIFFCUM93))} = \underset{(0.11)}{0.55} - \underset{(0.22)^{ns}}{0.14 \cdot \text{DIFN}}$	20	0.03
$\text{ARCSIN(SQRT(DIFF93))} = \underset{(0.09)}{0.88 \cdot X_{13}} - \underset{(0.18)^{ns}}{0.31 \cdot \text{DIFN}}$	20	0.09
$\text{ARCSIN(SQRT(DIFFCUM94))} = \underset{(0.06)}{0.67 \cdot X_{12}} + \underset{(0.08)}{0.34 \cdot X_3} + \underset{(0.12)}{0.27 \cdot \text{DIFN}}$	30	0.41

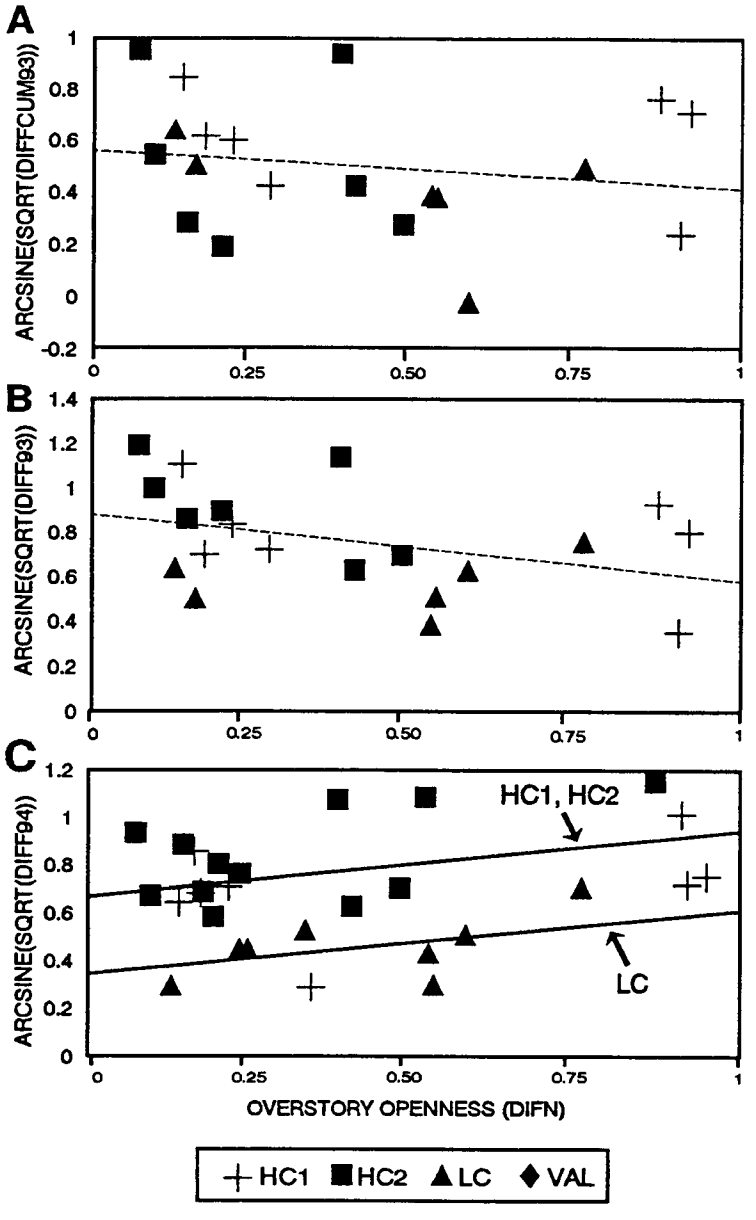

Figure 4.5 Regressions of the effect of bagging to exclude vertebrates versus overstory openness. **A.** DIFFCUM93=(Seed Development Efficiency for bagged branches)-(cumulative Seed Development Efficiency on unbagged branches), where cumulative Seed Development Efficiency includes all mature seeds counted in all 1993 censuses. **B.** DIFF93= (Seed Development Efficiency on bagged branches)-(Seed Development Efficiency on unbagged branches), where only September 1993 censuses are included. **C.** DIFF94=(Seed Development Efficiency on bagged branches)- (Seed Development Efficiency on unbagged branches), where only September 1994 censuses are included. Broken lines have slopes that are not significantly different from 0. Equations for regression lines are in Table 4.8.

Table 4.9 Analysis of carryover effects of bagging to exclude vertebrates in 1993 for Ovule Production, Seed Efficiency and growth on bagged branches in 1994. 1994 Growth- dry mass of all vegetative material distal to 1994 bud scars, measured in September 1994. See table 4.1 for other definitions.

Treatment	1994 Ovule Production	1994 Seed Efficiency	1994 Growth (g)
Bagged in 1993	13.02	0.58	0.78
Not Bagged in 1993	11.39	0.53	0.90
p*	0.60	0.50	0.53

*- Wilcoxon Signed-Rank comparisons between Bagged and Unbagged in 1993, probability of greater W, two-tailed. N=19 for all comparisons.

in the bagging experiment in 1993 at this site. Proportions of undeveloped seeds were used in these analyses rather than Seed Development Efficiency because Seed Development Efficiency was affected by predation as well as abortion.

Other Causes of Seed Abortion

Over 300 aborted female strobili were collected in 1993 for examination. 63% of these strobili were infested with phytophagous mites, the most common of which were *Cecidophyopsis psilaspis* Nalepa (Acari: Eriophyidae) and *Pentamerismus taxi* Haller (Acari: Tenuipalpidae). The extent to which these mites destroy developing strobili is unclear (Newsom 1993). However, 100 fully developed strobili and 100 aborted strobili were examined in 1994, and 90% of the healthy strobili were infested with mites, while

Table 4.10 Regression equations for the relationship between ovule and Seed Production between years and with growth variables. OVPROD94- Ovule Production in 1994; SDDVEF93BG- Seed Development Efficiency in 1993 on branches bagged with predator exclusion bags in 1993; SDDVEF94BG- Seed Development Efficiency in 1994 on branches bagged with predator exclusion bags both in 1993 and 1994; SDDVEF94- Seed Development Efficiency in 1994 on branches bagged with predator exclusion bags in 1993 (includes branches bagged and not bagged in 1994); PCTUND93BG- proportion of undeveloped seeds to Ovule Development on bagged branches in 1993; PCTUND94BG- proportion of undeveloped seeds to Ovule Development on bagged branches in 1994; PCTUND93- proportion of undeveloped seeds to Developoing Ovules on unbagged branches in 1993; INCR5YR- width of most recent 5 years of basal increment (mm); GROWTH94- mass of 1994 growth tips on sampled branches (g). Standard errors for parameter estimates are in parentheses below each estimate. All other abbreviations are as described in Table 4.5.

Model	df	R^2
$LN(OVPROD94) = \underset{(0.44)}{2.95} - \underset{(0.74)^{ns}}{0.82 \cdot SDDVEF93BG}$	24	0.01
$ARCSIN(SQRT(SDDVEF94BG)) = \underset{(0.12)}{0.32} + \underset{(0.18)}{0.46 \cdot ARCSIN(SQRT(SDDVEF93BG)}$	19	0.23
$ARCSIN(SQRT(PCTUND93BG)) = \underset{(0.12)}{0.28} + \underset{(0.016)^{ns}}{0.032 \cdot INCR5YR}$	24	0.11
$ARCSIN(SQRT(PCTUND94BG)) = \underset{(0.05)}{0.34} + \underset{(0.008)^{ns}}{0.013 \cdot INCR5YR}$	38	0.03
$ARCSIN(SQRT(PCTUND93BG)) = \underset{(0.12)}{0.32} + \underset{(0.11)^{ns}}{0.15 \cdot GROWTH94}$	24	0.03
$ARCSIN(SQRT(PCTUND94BG)) = \underset{(0.06)}{0.44 \cdot X_{134}} + \underset{(0.05)}{0.27 \cdot X_2} + \underset{(0.04)^{ns}}{0.053 \cdot GROWTH94}$	38	0.24
$ARCSIN(SQRT(PCTUND93)) = \underset{(0.07)}{0.63 \cdot X_{12}} + \underset{(0.08)}{0.30 \cdot X_{34}} - \underset{(0.01)^{ns}}{0.002 \cdot INCR5YR}$	50	0.25
$ARCSIN(SQRT(PCTUND93)) = \underset{(0.08)}{0.68 \cdot X_{12}} + \underset{(0.08)}{0.38 \cdot X_3} - \underset{(0.14)^{ns}}{0.087 \cdot X_4} - \underset{(0.077)^{ns}}{0.13 \cdot GROWTH94 \cdot X_{123}} + \underset{(0.08)}{0.25 \cdot GROWTH94 \cdot X_4}$	50	0.39

less than 50% of the aborted strobili were infested. This suggests that strobili can develop despite infestation by mites.

Approximately 25% of the aborted strobili examined in 1993 showed signs of fungal infection, but it was unknown whether infection occurred prior to abortion, or if there was a causal relationship. Other aborted strobili showed some evidence of frost damage, though this was not quantified. Finally, a large proportion of aborted strobili showed no gross pathology: it appeared that the megagametophyte ceased development and atrophied.

Variation Among Sites and Years

Early phase attrition (due at least in part to unsuccessful pollination) was significantly different among all four sites in both years of the study, accounting for the majority of attrition at VAL and LC, but for less than half of the observed attrition at HC1 and HC2 (Table 4.2, Figure 4.6). In contrast, predation accounted for more than half of the attrition at HC2 in 1993 and 1994, while it was a relatively minor source of attrition at LC and VAL in those years (Figure 4.6).

The patterns also varied somewhat between years. For example, Seed Development Efficiency was significantly higher in 1994 than in 1993 at LC and VAL but higher in 1993 at HC2 and not significantly different between years at HC1 (Table 4.2). However, Seed Efficiency did not differ between years except at site LC (Table 4.2), indicating that significant improvements in Ovule Development did not consistently translate into large improvements in Seed Production.

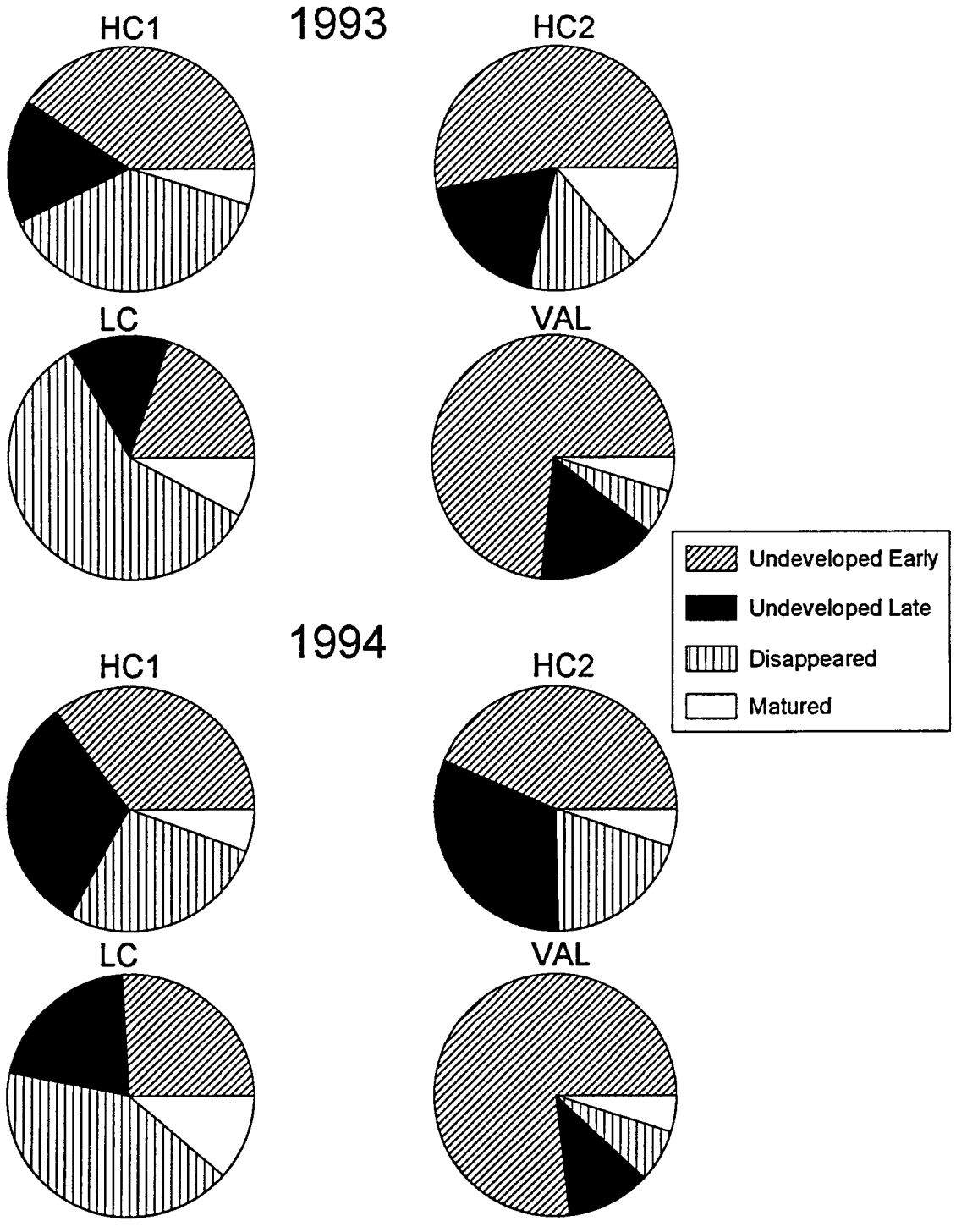

Figure 4.6 Fates of ovules. Undeveloped Early- Ovules that did not begin expanding by July. Undeveloped Late- ovules that began expanding before July but subsequently ceased developing; Disappeared- developing Ovules that disappeared between censuses, presumably due to predation. Matured- ovules that developed into mature seed.

DISCUSSION

There was significant attrition of ovules and developing seeds for *T. brevifolia* trees in western Oregon. Some of the factors that were associated with this attrition were the level of pollination success, the amount of available resources (as indexed by overstory canopy) and predation of developing seeds by vertebrates. These factors appeared to interact, and the importance of each factor varied at different sites and in different years.

Early Phase Attrition

There was significant attrition of ovules following the pollination period, with site averages ranging from 24 to 91% attrition (Table 4.2). Much of this attrition was probably due to lack of pollination. Exclusion of pollen almost always resulted in failure of ovules to develop, and the morphology of ovules from which pollen was excluded was indistinguishable from that of the majority of undeveloped ovules on unbagged branches during the same period (personal observation).

Supplemental pollination resulted in approximately doubled mean Seed Efficiency per tree at both sites. It is interesting to note that Seed Efficiency was significantly improved but Ovule Development was not significantly affected by supplemental pollination at site HC1. This may have been due to confounding effects of mite infestation at this site. For example, tree 14 had higher Ovule Development on untreated branches but approximately equal Seed Efficiency on supplemental pollination and untreated branches (Figure 4.1). This tree had an unusually severe

case of mite infestation (*P. taxi* and *C. psilaspis*), which resulted in swelling of infested buds that could have confounded assessment of Ovule Development (*i.e.* unpollinated but swollen ovules could potentially be mistaken for developing seeds). The number of seeds mistakenly characterized as developing might have been higher on control branches by chance, as the difference between control and pollen-supplemented branches was not significant.

Early phase attrition might have been caused by factors other than failed pollination. For example, ovules could have been damaged by insects, frost or pathogens. Seed Efficiency was not increased beyond 80% by hand-pollination in most cases, even with five hand-pollinations over a three week period. Also, Ovule Development was not significantly improved by supplemental pollination on 4 of 10 trees.

Methodological problems might also explain the failure of some supplementally pollinated ovules to develop. These include lack of receptivity at the time of pollen application, incompatibility with pollen used in pollen supplementation, clogging of the micropyles with heavy pollen loads, or physical damage to the ovules resulting from pollen application (Young and Young 1992).

Ovule development of *T. canadensis* was strongly associated with average nearest neighbor distance and average pollen production within a population (Allison 1990b). In the present study ovule development was not consistently associated with male density or average pollen production. This discrepancy could result from differences in the way male density was indexed, as Allison's (1990b)

index incorporated more spatial information than the index used here. Also, Allison's (1990b) correlations could be somewhat inflated, as he treated data from different years in the same population as independent data points (Snedechor and Cochran 1967).

The complexity of the relationship between male density and Ovule Development Efficiency observed in the present study can probably be attributed to several factors. One possibility is that 30 m was not the optimal radius to measure male density in. This number was not based on empirical measures of pollen flow. In fact, it appears that pollen travels much further than 30 m on most sites, as suggested by the positive y-intercepts for the regression equations. Another possibility is that Ovule Development Efficiency reflects sources of attrition other than failed pollination (see above), and the relative importance of different sources might have varied between sites and years. Even if differences in Ovule Development Efficiency were due primarily to pollination failure, variation among sites in the importance of male density could have been related to weather conditions at the time of pollination, proximity of compatible trees, quality of the pollen produced by the male trees, and synchrony of pollen shedding and female receptivity (Whitehead 1983). In particular, environmental cues for pollen shedding might have been more variable or ambiguous at sites with low pollination success. The two sites with the lowest Ovule Development, VAL and LC, had greater asynchrony in pollination than the higher sites, as demonstrated by significantly longer pollination periods (Chapter 2).

The overall importance of pollen in causing low seed-ovule ratios for *T. brevifolia* should not be overstated. Even in cases where Seed Efficiency was doubled by pollen application, a very low percentage of initiated ovules actually developed into seed (Figure 4.1B). Factors operating in the post-pollination period may help explain low Seed Efficiencies.

Overstory

The relationship between overstory openness and Ovule Development followed the same pattern reported previously for ovule Production (Chapter 3). Ovule and Seed Efficiency were positively associated with DIFN at all sites in both years except for HC1 in 1994. This positive relationship was probably due in part to correlation of resource availability with overstory canopy cover. The lack of a positive relationship at HC1 was due to lower Ovule Production in the OPN plot in 1994 as compared to 1993. This may have been due to harsh winter conditions that caused considerable pre-anthesis mortality of strobili (Chapter 3).

The positive correlation of Ovule Development with DIFN suggests that resource availability was important in setting the upper limit on Seed Efficiency in these *T. brevifolia* populations. However, caution should be exercised in drawing conclusions about the mechanisms of the effect of overstory removal on Ovule Production. Although light is the most obvious resource affected by canopy removal, the microenvironment of the understory is drastically changed in a variety of ways, and underground competition for nutrients

and water is likely to be strongly affected as well (Horn 1971). It is also possible that increased strobilus production under open canopy conditions was unrelated to resource levels, and was a physiological response to increased light only.

The results of this study contrast somewhat with those of related studies, as no relationship was found between Seed Efficiency and overstory openness (Figure 4.3C,D). Shading has been shown to limit flowering and seed production in a number of plant species (Dale and Causton 1992; Devlin 1988; Niesenbaum 1993; Owens and Blake 1985; Schutte Dahlem and Boerner 1987; Silen 1973). However, Crawford (1983) found that *T. brevifolia* trees in Idaho had *reduced* seed production following overstory removal compared to control trees under intact canopy. It is possible that *T. brevifolia* requires several years to equilibrate following overstory removal. Crawford (1983) examined trees in the year following overstory while trees were examined 3-30 years after overstory removal in the present study. However, Ovule Production and growth were both positively correlated with overstory openness in the present study (Chapter 3), suggesting that tree vigor increases with overstory openness.

The lack of a relationship between Seed Production was due in part to a negative correlation between Seed Efficiency and overstory openness. Pollination success cannot account for the lack of a relationship between DIFN and Seed Production because the number of initiated seeds was strongly correlated with DIFN. Therefore, post-pollination sources of attrition must have been responsible.

Late Phase Attrition

Bagging of branches to exclude vertebrates significantly improved Seed Development Efficiency at 3 of 4 sites in 1993 and 1994, indicating that predispersal seed predation was a significant source of late-phase seed attrition. However, considerable abortion of seeds occurred on branches bagged to exclude vertebrates, so factors other than predation are also important in seed abortion.

Little evidence was found of resource limitation of Seed Development Efficiency, as no trade-offs were detected between years in Seed Efficiency, between seed abortion and branch growth, or between seed abortion and 5-year stem increment. Also, no carryover effects of vertebrate exclusion were detected between years. If manipulated branch units were largely autonomous integrated physiological units (Casper and Niesenbaum 1993), and stored reserves played an important role in Seed Efficiency, then one might expect that a year of elevated Seed Efficiency would be followed by relatively low production (Sweet 1973), or that there might be a reduction in vegetative growth (Harper 1977) in the same year. Failure to detect such correlations could indicate that resource availability did not limit Seed Development Efficiency. An alternative explanation is that resource shortfalls on manipulated branches were compensated for by the shunting of resources from other parts of the tree (Janzen *et al.* 1980; Zimmerman and Pyke 1988). The amount of vegetative material covered by predator-exclusion bags represented a small proportion of the total plant (usually less than 10%), so increased seed production on these branches probably did not represent a

large increase in the total resource requirements of the tree. However, the extent to which resource shunting occurs in trees is unclear, and it is possible that individual branches are semi-autonomous Integrated Physiological Units (Casper and Niesenbaum 1993; Horvitz and Schemske 1988). Further evidence for autonomy among branches was provided by the pollen supplementation experiment, in which more than twice as many branches were manipulated as in the vertebrate-exclusion experiment, but there was no evidence of compromised Seed Efficiency on control branches.

It is important to note that lack of evidence of trade-offs in seed production and growth or between years does not constitute proof that resource limitation did not contribute to seed abortion. Reproductive growth might not be limited by the same resources as vegetative growth, or reproductive growth might depend entirely on current and local photosynthesis (Fox and Stevens 1991). Experimental manipulation of resource levels would provide a more definitive answer to the question of resource limitation of reproduction.

Other explanations for seed abortion include failed fertilization, genetic load, damage by pathogens, or spontaneous ovule abortion (*i.e.* a physiological process independent of resource availability or damage; see Ganeshaia and Uma Shaanker 1994). None of these possibilities were explored in the present study. It is also possible that the trees always produce more ovules than can be matured under prevailing conditions, and that these 'excess' ovules are destined to be aborted. The possible

evolutionary significance of excess ovules is discussed below.

Interactions Among Sources of Mortality

All of the major sources of ovule attrition probably interacted in some way. For example, overstory openness was not associated with Seed Production on unbagged branches, but was positively associated with Seed Production on branches bagged to exclude vertebrates. Regressions of bagging effect versus DIFN were significant and positive 1994 but not in 1993. This discrepancy between years may reflect different effects of canopy cover on animal activity between the two years. An alternative explanation is that the combination of bagging and overstory canopy caused a reduction in photosynthesis that prevented seed development. Perhaps this effect was only detected in 1994 because of residual effects of 1993 bagging. Evidence against this explanation is the lack of significant differences in 1994 Ovule Production, Seed Development Efficiency and branch growth on branches bagged in 1993. Also, the study design, which provided for an even split of branches bagged in 1993 between bagged and control groups in 1994, should have accounted for possible carryover effects from 1993. A third explanation for the difference in the effect of overstory on bagging effect in the two years is that some factor such as resource availability was differentially important during the two years of the study. One might expect bagging to be more effective under high resource conditions, because a larger proportion of filled seed would be available to predators (Ehrlén 1992). If a resource (e.g. water, light)

positively associated with overstory openness was limiting in 1994 and not in 1993, then this would explain why the effect of bagging was only associated with overstory in 1994. However, the limiting resources and mechanisms of late season ovule abortion are not known, so this question can't be answered definitively.

Interactions between predation, pollination and other sources of late season attrition were also pronounced. For example, the large discrepancy between Ovule Development and Seed Efficiency at all sites was probably due in large part to predation. Even in situations were pollination success was unusually high, the bulk of excess seeds would probably be destroyed by predators, or fail to develop because of resource limitation or genetic load, as shown by the pollen supplementation experiments (Figure 4.2).

This study points out the importance of considering several potential sources of attrition simultaneously at several distinct sites over several years. Few studies have taken the effects of predation into account when examining the importance of pollen and resource limitation. A common practice has been to exclude from consideration all fruits or seeds that showed evidence of predation (e.g. Campbell and Halama 1993; Gorchov 1988; Vaughton 1991; Zimmerman and Pyke 1988; but see Ehrlén 1992). It is also important to be cautious in drawing broad conclusions from narrowly focused studies performed at a few similar sites under relatively uniform conditions. Limitations to seed production can vary markedly from site to site and year to year. For example, if the present study had been performed solely at site HC2, the conclusions about the relative importance of pollen

versus predation in accounting for low seed-ovule ratios would have been quite different than if the study had been performed exclusively at VAL.

Evolutionary Hypotheses

It appears that low Seed Efficiency is a common occurrence for *T. brevifolia*. The evolutionary significance of low seed-ovule ratios has been discussed extensively over the past 15 years (for reviews see Bawa and Webb 1984; Ehrlén 1991; Stephenson 1981; Sutherland 1986). It is useful to review some of these theories as they relate to dioecious, wind-pollinated trees, as they provide insights into the evolutionary forces that favor low seed efficiency.

Sexual selection is one force that could favor low seed-ovule ratios. Sexual selection results from the differential mating success of members of one sex with the opposite sex (Arnold 1994). Sexual selection in plants is believed to result from competition among pollen donors for receptive stigmas and consistent discrimination among pollen haplotypes by females (female choice: Snow 1994; Stephenson and Bertin 1983; Willson and Burley 1983; but see Queller 1994). Low seed-ovule ratios would result if female choice operated through selective abortion or development of seeds (Stephenson 1981; Sutherland 1986). Sexual dimorphisms such as high pollen:ovule ratios and low pollen:ovule mass ratios indicate that sexual selection could be a dominant force in evolution (Willson 1979). For plants in which sexual selection was important, pollen limitation is expected to be relatively unimportant because male plants should produce excess pollen in competing for mates, while female plants

should be limited primarily by the availability of resources to provision seeds (e.g. Willson and Burley 1983).

Another hypothesis about the significance of excess ovules concerns uncertainty in levels of resource availability, pollen availability or predation over time ('ecological window' hypothesis, Ehrlén 1991), so that ovules that normally abort will develop into seeds under unusually favorable conditions (Aker 1982; Bawa and Webb 1984; Janzen *et al.* 1980; Lloyd 1980; Udovic 1981). For plants in which differentiation of reproductive buds occurs well before pollination, one would expect excess ovules to be important because ecological windows would be particularly unpredictable (Lloyd 1980), and the number of ovules produced sets the upper limit on seed production.

A related hypothesis is that excess ovules serve as 'reserve ovaries' to compensate for sources of mortality that are beyond maternal control, but relatively constant (Ehrlén 1991). Some assumptions behind this theory are that the investment in excess ovules is balanced by the cost of missed reproductive opportunities, and that the effect is likely to be most pronounced for plants in which ovules are cheap to produce relative to fruits (Ehrlén 1991).

All three of these theories could be functioning simultaneously in favoring low seed-ovule ratios in *T. brevifolia*. *T. brevifolia* fits the profile of plants for which sexual selection has been important. Male trees produce many more strobili than females, and pollen-ovule ratios are greater than 1,000,000:1 (Chapter 2). However, it does appear that pollen limitation of seed production occurs at least at some sites for some trees. Burd (1994)

pointed out that the regular occurrence of pollen limitation does not necessarily contraindicate the importance of sexual selection. This is because of stochasticity inherent in the pollination process, so that even if male-male competition is intense (*i.e.* high pollen-ovule ratios), male gametes may fail to encounter receptive females, and female choice may not occur. A similar case can be made for stochasticity in other factors affecting seed abortion, such as predation. In situations of high predation the number of developing seeds may fall below the number that could be matured with available resources, and female choice could be severely restricted (Niesenbaum and Casper 1994; Ehrlén 1992). Thus, the importance of female choice can depend on windows of ecological opportunity.

Ecological windows in predation pressure could be particularly important force in determining seed-ovule ratios in *T. brevifolia*. The effect of predation on seed production was especially pronounced at 3 of 4 sites examined in this study. Predispersal seed predators have great potential to influence the evolutionary history of a plant (Smith 1971) and some tree species adapt to severe predation by 'mast seeding' in which population seed production is synchronous and concentrated in certain years (Janzen 1973). This is believed to result in predator satiation during mast years, and/or predator starvation in non-mast years (Silvertown 1980). There was no evidence of masting by *T. brevifolia* during four years of observation at these four sites (Vance, personal communication). In order for masting to be effective, the tree seed must constitute a significant portion of the diet of the predator (Silvertown

1980). *T. brevifolia* seed production is a fraction of that of dominant overstory conifers. Therefore, it is likely that fluctuations in predator abundance occur independently of the amount of *T. brevifolia* seed production. This would favor a 'bet-hedging' reproductive strategy because excess ovule production would translate into high seed production during unpredictable ecological windows when predation levels were low.

If the reserve ovary hypothesis were important for *T. brevifolia*, seed efficiencies would be low even in the absence of predation or with excess pollen, because ovule production would be in excess of that which could be matured with existing resources (Ehrlén 1991; Guitian 1993). In the present study, even in cases of high pollination where predators were excluded (e.g. site HC2, bagged branches), nearly half of the ovules initiated failed to develop into mature seeds. In fact, in no case did average Seed Efficiency per tree exceed 70%, regardless of treatment. Under the conditions examined in this study, it appears that a certain level of abortion is likely to occur regardless of pollination conditions or levels of predation. If this abortion is due to resource limitation, it may be explained by a poor correspondence between the level of resource availability and ovule production (Lloyd 1980), or it could be explained by the reserve ovary hypothesis. Perhaps in the case of *T. brevifolia* a certain proportion of ovules initiated is doomed to failure because of inefficiencies inherent in wind-pollination (Whitehead 1983), because outcrossing species typically suffer from high genetic load (Charlesworth 1989), or because of spontaneous abortion due

to random self-organization of developing ovules (Ganeshaiah and Uma Shaanker 1994). In such a case plants that produced excess ovules would be at a selective advantage if the final number of seeds closely matched available resources.

Of course, more careful study would be required to distinguish among the many possible evolutionary explanations for excess ovule production. Controlled pollinations with known parents or paternity analysis would be required to determine the importance of sexual selection (Willson and Burley 1983; Snow 1994). Experiments manipulating resource availability would be required to establish more clearly the role of resources in limiting seed production in the absence of predation. The level of genetic load could be determined by comparing seed and seedling characteristics of selfed progeny (using cosexual *T. brevifolia* trees, Chapter 2) to outcrossed progeny (Sorensen 1969). Long-term studies are necessary to determine the degree to which seed efficiencies are constant and how much they are affected by variation in weather patterns and abundance of predators.

CHAPTER 5 CONCLUSIONS

Sexual reproduction probably plays an important role in determining the distribution, abundance and genetic diversity of wild *T. brevifolia* populations. *T. brevifolia* is easily destroyed by fire, which occurs periodically throughout its range. Limitations on sexual reproduction may cause *T. brevifolia* to be restricted primarily to old-growth forests.

This thesis explored some of the ecological factors that influenced seed production of *T. brevifolia* on four sites in western Oregon. Overstory canopy openness was correlated with many aspects of sexual reproduction. Phenology of pollen shedding and seed development was associated with overstory openness. Open-grown trees tended to shed pollen earlier and, in some cases, mature seeds earlier, than trees growing under intact canopy on the same site. Overstory openness was also positively associated with reproductive potential, the number of male and female strobili produced per branch. However, Seed Efficiency, the proportion of ovules that developed into mature seeds, was negatively associated with overstory openness, and there was no overall association between Seed Production and overstory openness.

The association of seed efficiency and overstory openness was probably due in part to variation in predation of seed by vertebrates under different overstory conditions. When vertebrates were excluded, seed production was positively associated with overstory openness. Also, the effects of bagging were negatively associated with overstory

openness in 1994. Inadequate pollination also affected Seed Efficiency, but this effect didn't vary systematically with overstory. Also, inadequate pollination did not appear to be a predominant factor limiting seed production, as some sites had low rates of early-season attrition, and supplemental pollination failed to improve Seed Efficiency beyond 10% on sites with high early attrition.

Temporal and spatial variation in factors affecting seed production was also explored. Female strobilus production was higher in 1993 than in 1994 on most sites, but seed production was not significantly different between years. This was due in part to lower Seed Efficiency in 1993. This difference was manifested both at the early stages in ovule development, presumably due to interannual variation in pollination efficiency, and at the later stages in ovule development.

Spatial variation was apparent both within sites, as illustrated by the effect of overstory openness, and among sites. In particular, there were several striking differences between the 3 Cascade mountain sites and the Willamette Valley site. The Willamette Valley trees began pollination earlier and pollen shedding was less synchronized than at the Cascade sites. Willamette Valley trees produced larger seeds, and displayed less cosexuality than Cascade trees. Relationships between various measures of sexual reproduction and overstory openness also differed consistently between Willamette Valley and Cascade sites. Intercepts were lower and slopes were steeper for the valley site, because few strobili and no seeds were observed on trees growing under overstory canopy at the Valley site,

while productivity of open-grown trees converged with that of the Cascade trees. Patterns of attrition differed as well. Ovule Development Efficiency was considerably lower at the Valley site than for the Cascade sites for both years of the study. Also, bagging to exclude predators did not significantly improve Seed Development Efficiency for Willamette Valley trees. This was due to high rates of seed abortion on bagged branches due to unknown causes.

Some of the differences between the Willamette Valley and Cascade sites were probably due to chance differences among the sites. Because only one Valley site was included in the study, little inference can be made about the behavior of all *T. brevifolia* populations in the Willamette Valley. For example, one of the reasons the slopes for regressions of strobilus and seed production versus overstory openness were greater for the valley site is the trees growing under overstory canopy were on a steep north-facing incline on this site. Potential solar radiation was low, and productivity was depressed. In contrast, most trees growing under overstory canopy at the Cascade sites were either on gentle slopes or southern aspects, so productivity was not as depressed. However, some of the differences between the Willamette Valley and the Cascades, such as higher seed weight and low Seed Efficiency on bagged branches, are probably associated with environmental differences between the sites. For example, rainfall in the Willamette valley is substantially lower than at the other three sites (Table 2.1), and there is virtually no snowfall in the winter in the valley. Other factors such as vegetation association and soil type were also quite

distinct between the valley and Cascade Mountain sites. Further study would be required to isolate the factors responsible for differences between the valley and Cascade sites.

This study demonstrated the considerable logistical difficulties involved in characterizing the reproductive ecology of a species. If the study had been performed in a single year or a single site, a limited and possibly erroneous description would have resulted. Similarly, if reproductive effort had been censused at a single point in time, conclusions about the effect of overstory would have differed if the census were performed in July or October. Finally, if the effects of overstory had been examined without considering the effects of predation or pollination, a limited understanding of factors affecting seed production would have emerged. Considerable time, effort and expense is required if the ecological forces that affect reproductive success are to be understood. However, this investment is necessary if the complex mechanisms of ecosystem function are to be understood.

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APPENDIX A DATA FROM INDIVIDUAL TREES

Table A.1 Characteristics of female trees. DIFN- overstory openness; AGE- number of annual increments; SIZE- $dbh^2 \cdot h$ (m^3); POTRAD- potential radiation ($cal/cm^2/yr.$), determined from slope and aspect measured at each tree; BRWT- average dry mass of sampled branch segments (g); SLA- specific leaf area (cm^2/g); 94GRTH- dry mass of branch material distal to 1994 bud scar (g); 5YRINCR- width of most recent 5 annual increments (mm); OVULE- number of ovules per 30 cm branch; SDU- 1994 Average seed production per 30 cm branch segment on unbagged branches. SDB- Average seed production per 30 cm branch segment on branches bagged to exclude predators. Codes are site codes followed by tree numbers and a letter indicating sex (F- female).

TREE	DIFN	AGE	SIZE	POTRAD	BRWT	SLA
hc101F	0.931	36	0.024	207741	6.43	1.49
hc102F	0.873	45	0.033	185347	9.27	2.32
hc103F	0.949	102	0.053	208454	10.53	2.96
hc104F	0.896	96	0.045	156839	10.71	.
hc105F	0.911	85	0.019	160143	6.77	2.65
hc106F	0.904	110	0.062	186561	11.00	1.75
hc111F	0.920	75	0.083	199538	11.54	1.98
hc113F	0.172	220	0.139	202440	4.69	2.07
hc114F	0.182	130	0.409	216489	5.35	2.44
hc118F	0.162	120	0.036	235104	3.80	1.66
hc120F	0.214	62	0.054	232955	3.39	2.34
hc123F	0.341	149	0.079	209913	4.54	3.36
hc126F	0.271	120	0.062	216821	3.87	2.16
hc127F	0.138	95	0.154	225198	5.23	1.61
lc01F	0.067	110	0.016	201653	5.70	2.02
lc02F	0.080	145	0.133	198185	3.68	2.16
lc03F	0.096	125	0.119	200429	3.66	.
lc04F	0.158	95	0.024	200021	3.51	2.84
lc05F	0.230	134	0.466	195618	2.02	1.86
lc06F	0.126	113	0.283	178402	4.35	1.98
lc07F	0.332	163	0.210	202351	4.41	2.56
lc11F	0.581	137	0.095	206023	7.01	2.44
lc16F	0.756	132	0.174	185454	7.56	2.49
lc23F	0.389	125	0.269	190412	5.94	2.96
lc25F	0.289	117	0.089	190490	3.07	2.66
lc28F	0.523	154	0.192	200182	2.01	2.99
lc30F	0.243	187	0.178	189313	2.86	1.49
lc33F	0.531	130	0.276	200784	2.32	1.89

Table A.1 (continued)

TREE	DIFN	AGE	VOL	POTRD	BRWT	SLA
hc201F	0.144	117	0.108	177737	2.81	2.20
hc202F	0.034	101	0.039	165078	1.45	2.16
hc203F	0.094	130	0.099	159719	2.29	2.63
hc204F	0.174	153	0.444	154583	1.76	2.79
hc205F	0.197	152	0.042	169293	2.16	2.53
hc206F	0.124	130	0.074	171914	2.45	.
hc207F	0.048	130	0.021	163380	1.67	3.29
hc211F	0.231	.	0.149	152030	.	.
hc212F	0.071	77	0.161	154900	2.20	3.04
hc213F	0.403	189	0.288	149371	2.69	2.32
hc214F	0.190	170	0.312	151821	3.58	2.81
hc215F	0.478	187	0.226	159330	3.07	2.32
hc216F	0.380	190	0.305	152614	2.48	1.91
hc217F	0.231	187	1.314	149153	2.93	2.17
hc223F	0.517	88	0.322	156098	1.55	2.26
hc224F	0.869	100	0.090	161996	4.69	1.62
val02F	0.924	75	1.285	187376	3.52	2.78
val05F	0.882	100	0.299	221316	2.50	3.11
val08F	0.691	65	0.480	212884	4.36	3.52
val12F	0.870	59	0.371	212237	2.43	3.06
val14F	0.832	85	0.579	216974	6.72	4.71
val17F	0.890	99	0.446	206791	3.38	2.91
val20F	0.732	77	0.199	213542	3.43	2.30
val111F	0.073	90	0.215	164737	0.72	.
val128F	0.053	73	0.072	207228	.	.
val130F	0.135	106	0.340	139128	1.84	.
val134F	0.136	67	0.072	215717	1.12	3.04
val153F	0.171	54	0.219	104239	1.52	3.94
val156F	0.129	58	0.445	126703	1.39	.
val171F	0.149	96	0.146	160510	1.66	3.39
val177F	0.449	97	1.340	223561	2.82	2.47

Table A.1 (continued)

TREE	94GRTH	5YRINCR	OVULE93	SDU93	SDB93
hc101F	0.73	4.5	28.87	0.75	.
hc102F	3.03	7.0	31.50	1.75	14.00
hc103F	1.57	9.7	30.75	4.12	.
hc104F	1.80	13.4	46.50	4.12	.
hc105F	1.25	12.0	20.12	2.00	.
hc106F	1.10	7.0	35.00	1.25	4.50
hc111F	1.16	8.2	34.25	5.25	18.00
hc113F	1.02	4.9	20.62	0.50	1.50
hc114F	0.26	4.1	48.00	7.75	.
hc118F	0.30	8.0	18.62	4.00	.
hc120F	0.51	3.9	16.75	3.25	11.00
hc123F	0.44	4.9	14.87	1.50	.
hc126F	0.56	9.2	19.00	6.50	11.25
hc127F	0.72	5.9	11.37	1.25	5.00
lc01F	0.85	1.2	1.00	0.25	.
lc02F	0.46	5.5	4.00	0.50	.
lc03F	0.37	2.7	7.37	1.60	3.00
lc04F	0.32	1.0	4.12	0.25	1.50
lc05F	0.14	1.8	4.00	0.37	.
lc06F	0.72	5.0	6.50	0.00	1.33
lc07F	0.49	3.3	4.62	0.50	.
lc11F	0.54	5.0	53.75	5.50	8.00
lc16F	1.35	12.1	33.37	2.50	14.75
lc23F	0.99	1.2	3.50	0.37	.
lc25F	0.33	4.0	2.25	0.25	.
lc28F	0.55	4.7	7.62	0.00	1.75
lc30F	0.99	2.8	3.37	0.00	.
lc33F	0.50	3.0	8.87	0.66	6.00
hc201F	1.12	1.0	26.37	7.50	10.00
hc202F	0.36	1.9	4.12	3.50	.
hc203F	0.73	2.8	13.12	4.00	6.25
hc204F	0.55	3.6	10.12	5.12	.
hc205F	0.87	1.5	9.00	6.25	4.25
hc206F	1.00	1.5	7.25	2.50	.
hc207F	0.77	3.0	3.75	1.00	.
hc211F	.	.	1.50	0.37	.
hc212F	0.91	10.0	25.00	1.50	28.75
hc213F	0.73	3.0	24.37	2.50	11.00
hc214F	1.03	2.3	14.87	2.00	.
hc215F	0.45	3.0	10.25	3.25	6.50
hc216F	0.72	1.1	12.62	0.60	17.33
hc217F	0.99	4.0	21.50	4.50	.

Table A.1 (continued)

TREE	94GRTH	5YRINCR	OVULE93	SDU93	SDB93
hc223F	0.62	7.2	22.12	0.87	.
hc224F	1.51	5.5	47.87	6.62	.
val02F	0.79	11.7	18.87	0.25	1.25
val05F	2.28	14.0	58.75	13.50	5.00
val08F	2.44	4.5	11.00	0.00	.
val12F	1.42	13.2	6.55	2.00	2.00
val14F	2.83	8.1	6.00	1.25	.
val17F	2.62	12.0	18.25	1.00	.
val20F	1.49	9.9	37.75	3.50	.
val111F	0.28	1.7	2.62	0.00	.
val128F	.	3.3	1.25	0.00	.
val130F	0.40	0.8	1.00	0.00	.
val134F	0.60	5.7	2.12	0.00	.
val153F	0.56	7.2	2.37	0.00	.
val156F	0.32	3.7	1.25	0.00	.
val171F	0.17	2.6	1.37	0.00	.
val177F	.	9.4	.	.	.

Table A.1 (continued)

TREE	OVULE94	SDU94	SDB94
hc101F	20.62	1.00	.
hc102F	20.62	1.25	.
hc103F	15.12	1.50	11.00
hc104F	30.75	3.00	11.00
hc105F	18.87	0.25	15.75
hc106F	14.62	0.75	.
hc111F	18.71	2.00	16.75
hc113F	22.37	2.40	6.66
hc114F	42.75	2.14	.
hc118F	6.25	0.00	4.25
hc120F	6.25	0.50	2.50
hc123F	9.25	0.80	6.66
hc126F	14.00	1.37	.
hc127F	12.62	3.00	2.75
lc01F	1.12	0.75	.
lc02F	2.50	0.87	.
lc03F	2.00	1.00	1.33
lc04F	1.25	0.87	.
lc05F	2.50	1.00	0.75
lc06F	6.57	1.00	3.00
lc07F	4.62	1.00	2.25
lc11F	36.62	4.25	5.75
lc16F	26.62	3.00	10.25
lc23F	2.00	0.12	.
lc25F	0.62	0.00	.
lc28F	7.37	1.00	2.75
lc30F	5.12	1.25	3.00
lc33F	10.12	2.00	5.75
hc201F	29.87	3.75	19.00
hc202F	2.75	1.00	3.00
hc203F	3.25	1.50	1.75
hc204F	6.25	2.75	6.50
hc205F	3.87	2.00	4.50
hc206F	6.87	2.00	4.00
hc207F	1.66	0.50	0.66
hc211F	.	.	.
hc212F	11.00	0.66	8.50
hc213F	34.62	3.50	13.75
hc214F	4.12	1.75	6.00
hc215F	10.50	2.50	4.75
hc216F	6.12	2.75	2.00
hc217F	23.87	1.75	15.75

Table A.1 (continued)

TREE	OVULE94	SDU94	SDB94
hc223F	21.50	1.50	21.75
hc224F	34.75	1.75	27.50
val02F	11.37	0.25	0.50
val05F	14.37	3.66	2.75
val08F	31.75	0.50	1.00
val12F	17.87	1.62	.
val14F	5.00	0.00	1.25
val17F	25.12	0.62	.
val20F	33.75	6.62	.
val111F	1.50	0.00	.
val128F	0.37	0.00	.
val130F	0.25	0.00	.
val134F	0.87	0.00	.
val153F	1.50	0.00	.
val156F	2.00	0.00	.
val171F	0.87	0.25	.
val177F	7.75	1.00	1.00

Table A.2 Characteristics of male trees. STROB- average number of male strobili produced per 20 cm branch segment. Codes are site codes followed by the tree number and a letter indicating sex (M- male). All other abbreviations are as described in Table A.1.

TREE	DIFN	AGE	SIZE	POTRAD	BRWT	5YRINCR	STROB93	STROB94
HC1001M	0.880	67	0.195	195582	3.92	3.7	93.0	134.2
HC1003M	0.826	60	0.021	200511	2.66	6.8	90.0	109.5
HC1004M	0.836	87	0.121	164591	4.96	6.7	70.0	83.7
HC1005M	0.799	115	0.141	165964	2.56	7.0	45.2	27.7
HC1006M	0.804	95	0.103	138951	3.31	5.0	74.2	61.5
HC1007M	0.857	230	0.433	137923	3.59	16.0	84.7	82.7
HC1008M	0.799	68	0.048	185847	2.56	6.2	25.5	19.2
HC1015M	0.114	116	0.103	223271	1.51	4.7	35.0	13.6
HC1018M	0.097	106	0.050	233620	1.56	1.9	28.0	20.0
HC1019M	0.192	76	0.069	235002	1.15	1.2	31.6	19.0
HC1023M	0.202	62	0.048	235104	1.21	4.7	31.7	17.1
HC1029M	0.111	95	0.159	227292	1.82	1.2	8.0	6.1
HC1033M	0.253	185	0.146	225921	1.85	3.7	53.1	39.0
HC1036M	0.182	125	0.318	223737	1.16	2.6	15.3	28.7
LC003M	0.071	95	0.123	201903	1.39	6.5	9.3	6.1
LC004M	0.081	187	0.353	203209	1.23	1.5	5.5	7.5
LC005M	0.112	132	0.038	198892	0.96	2.0	4.2	6.8
LC007M	0.088	125	0.095	202581	1.33	7.0	1.8	2.2
LC008M	0.153	185	0.073	196855	1.17	1.8	1.0	1.7
LC009M	0.264	199	0.248	200021	1.28	1.0	9.8	8.8
LC011M	0.128	172	0.105	193325	1.24	2.8	14.3	24.2
LC018M	0.484	110	0.227	206695	1.85	6.8	.	30.8
LC026M	0.882	195	0.303	212133	3.61	6.3	52.0	142.3
LC029M	0.830	35	0.266	201259	2.72	6.8	33.2	30.6
LC031M	0.362	205	0.212	197316	1.53	3.4	17.8	10.7
LC035M	0.424	133	0.269	162136	1.97	1.4	3.1	10.5
LC036M	0.161	185	0.405	202534	2.31	3.6	0.6	1.7
LC042M	0.540	269	0.961	210996	2.95	3.7	40.5	64.8
LC046M	0.462	100	0.260	228012	2.10	6.8	14.3	13.5
HC2001M	0.058	140	0.164	173845	1.31	2.8	14.6	5.2
HC2002M	0.217	161	0.043	173293	1.06	0.9	7.8	9.1
HC2003M	0.121	149	0.081	159719	1.52	1.6	28.5	28.8
HC2004M	0.044	215	0.058	177386	1.23	0.7	8.7	6.1
HC2006M	0.023	179	0.166	174009	1.67	2.3	18.2	13.2
HC2007M	0.166	63	0.030	161183	2.37	4.5	24.2	12.2
HC2011M	0.059	220	0.099	179806	1.48	1.8	21.5	13.5

Table A.2 (continued)

TREE	DIFN	AGE	SIZE	POTRAD	BRWT	5YRINCR	STROB93	STROB94
HC24101M	0.292	165	0.304	148709	3.25	4.0	90.1	86.8
HC24102M	0.332	208	0.313	151193	2.14	4.7	51.1	42.5
HC24103M	0.200	177	0.324	165100	2.47	4.9	33.2	63.6
HC24104M	0.392	167	1.034	158315	2.66	1.5	77.8	26.5
HC24105M	0.323	112	0.348	159330	2.96	3.0	30.7	35.7
HC24106M	0.501	125	0.280	158189	1.52	6.0	28.2	27.8
HC24107M	0.387	110	0.135	150938	2.93	5.3	22.1	22.3
HC2411M	0.965	105	0.495	186331	4.26	5.9	117.2	139.8
HC2413M	0.990	215	0.670	168636	3.75	4.3	63.5	96.6
VAL007M	0.635	80	0.196	188581	2.72	7.5	75.5	59.5
VAL015M	0.847	78	0.143	211589	5.44	14.5	85.5	29.5
VAL016M	0.839	83	0.135	222221	5.02	14.20	44.1	66.8
VAL021M	0.881	137	1.353	222219	5.37	7.80	48.0	133.6
VAL033M	0.823	120	0.521	138807	5.04	10.60	35.5	76.6
VAL035M	0.898	125	0.581	196243	4.74	7.50	43.3	58.7
VAL036M	0.914	87	1.542	202618	3.75	8.60	45.8	89.1
VAL060M	0.107	69	0.201	174003	1.43	1.90	6.7	9.8
VAL073M	0.111	113	0.503	172470	2.29	1.70	8.8	14.8
VAL076M	0.054	75	0.123	164737	1.33	1.60	1.0	1.0
VAL102M	0.225	50	0.192	148759	1.90	6.60	11.3	10.1
VAL110M	0.100	84	0.069	157047	1.91	4.40	4.5	6.6
VAL112M	0.061	104	0.436	167874	1.52	0.96	11.3	12.2
VAL118M	0.055	59	0.044	181847	1.67	4.50	3.7	5.2
VAL122M	0.042	67	0.191	173991	1.72	1.00	2.5	3.6
VAL131M	0.190	75	0.373	139128	.	1.20	4.3	.
VAL152M	0.087	60	0.098	140568	1.15	3.00	0.7	0.1
VAL157M	0.043	77	0.077	130739	1.85	1.10	3.2	4.6
VAL158M	0.055	85	0.191	147752	1.20	0.80	8.0	16.5
VAL159M	0.052	70	0.045	176920	1.05	0.90	4.2	10.1
VAL160M	0.103	91	0.127	204402	1.37	0.60	6.1	5.6
VAL169M	0.120	57	0.177	143276	2.13	5.00	2.1	22.2
VAL176M	0.561	88	0.555	182232	1.94	4.20	.	9.7

APPENDIX B CORRELATION MATRICES

Table B.1 Pearson correlation coefficients for variables measured for female trees, all sites grouped. Line 1- Correlation coefficient; Line 2- Probability of greater |R|, under H_0: $\rho=0$; Line 3- number of trees. All other abbreviations are as described in Table A.1.

	DIFN	LGINCR5	INVSLA	FOLBR
DIFN	1.00000	0.62174	0.58711	0.25198
	0.0	0.0001	0.0001	0.0610
	58	58	51	56
LGINCR5	0.62174	1.00000	0.32791	0.00406
	0.0001	0.0	0.0188	0.9763
	58	58	51	56
INVSLA	0.58711	0.32791	1.00000	0.25897
	0.0001	0.0188	0.0	0.0694
	51	51	51	50
FOLBR	0.25198	0.00406	0.25897	1.00000
	0.0610	0.9763	0.0694	0.0
	56	56	50	56
BRWT	0.68723	0.37441	0.59013	-0.01267
	0.0001	0.0045	0.0001	0.9261
	56	56	50	56
AGE	-0.30785	-0.34860	-0.06800	-0.33173
	0.0187	0.0073	0.6354	0.0125
	58	58	51	56
SIZE	0.12209	0.16620	0.07399	0.08941
	0.3613	0.2124	0.6059	0.5123
	58	58	51	56
POTRAD	0.21522	0.28256	0.08917	0.21949
	0.1047	0.0316	0.5338	0.1041
	58	58	51	56

Table B.1 (continued)

	BRWT	AGE	SIZE	POTRAD
DIFN	0.68723	-0.30785	0.12209	0.21522
	0.0001	0.0187	0.3613	0.1047
	56	58	58	58
LGINCR5	0.37441	-0.34860	0.16620	0.28256
	0.0045	0.0073	0.2124	0.0316
	56	58	58	58
INVSLA	0.59013	-0.06800	0.07399	0.08917
	0.0001	0.6354	0.6059	0.5338
	50	51	51	51
FOLBR	-0.01267	-0.33173	0.08941	0.21949
	0.9261	0.0125	0.5123	0.1041
	56	56	56	56
BRWT	1.00000	-0.06795	0.06204	0.02339
	0.0	0.6188	0.6497	0.8641
	56	56	56	56
AGE	-0.06795	1.00000	0.06068	-0.13801
	0.6188	0.0	0.6509	0.3015
	56	58	58	58
SIZE	0.06204	0.06068	1.00000	-0.04425
	0.6497	0.6509	0.0	0.7415
	56	58	58	58
POTRAD	0.02339	-0.13801	-0.04425	1.00000
	0.8641	0.3015	0.7415	0.0
	56	58	58	58

Table B.2 Pearson correlation coefficients for variables measured for male trees, all sites grouped. Line 1- Correlation coefficients; Line 2- Probability of greater |R|, under H_0: $\rho=0$; Line 3- number of trees.

	DIFN	LGINCR5	FOLBR	BRWT
DIFN	1.00000	0.65047	-0.04955	0.84560
	0.0	0.0001	0.7070	0.0001
	61	61	60	60
LGINCR5	0.65047	1.00000	-0.03418	0.65241
	0.0001	0.0	0.7954	0.0001
	61	61	60	60
FOLBR	-0.04955	-0.03418	1.00000	-0.03364
	0.7070	0.7954	0.0	0.7986
	60	60	60	60
BRWT	0.84560	0.65241	-0.03364	1.00000
	0.0001	0.0001	0.7986	0.0
	60	60	60	60
AGE	0.05709	-0.11746	0.16171	0.00068
	0.6621	0.3673	0.2171	0.9959
	61	61	60	60
SIZE	0.57819	0.25138	0.04048	0.55291
	0.0001	0.0507	0.7588	0.0001
	61	61	60	60
POTRAD	0.18616	0.17741	0.04627	0.03822
	0.1509	0.1714	0.7256	0.7719
	61	61	60	60

Table B.2 (continued)

	AGE	SIZE	POTRAD
DIFN	0.05709	0.57819	0.18616
	0.6621	0.0001	0.1509
	61	61	61
LGINCR5	-0.11746	0.25138	0.17741
	0.3673	0.0507	0.1714
	61	61	61
FOLBR	0.16171	0.04048	0.04627
	0.2171	0.7588	0.7256
	60	60	60
BRWT	0.00068	0.55291	0.03822
	0.9959	0.0001	0.7719
	60	60	60
AGE	1.00000	0.20961	0.10134
	0.0	0.1049	0.4371
	61	61	61
SIZE	0.20961	1.00000	0.04776
	0.1049	0.0	0.7147
	61	61	61
POTRAD	0.10134	0.04776	1.00000
	0.4371	0.7147	0.0
	61	61	61

Table B.3 Pearson correlation coefficients for variables measured for all trees, all sites grouped. Line 1- Correlation coefficients; Line 2- Probability of greater |R|, under H_0: $\rho=0$; Line 3- number of trees.

	DIFN	LGINCR5	INVSLA	FOLBR
DIFN	1.00000	0.65361	0.58711	-0.02001
	0.0	0.0001	0.0001	0.8261
	126	126	51	123
LGINCR5	0.65361	1.00000	0.32791	-0.13854
	0.0001	0.0	0.0188	0.1265
	126	126	51	123
INVSLA	0.58711	0.32791	1.00000	0.25897
	0.0001	0.0188	0.0	0.0694
	51	51	51	50
FOLBR	-0.02001	-0.13854	0.25897	1.00000
	0.8261	0.1265	0.0694	0.0
	123	123	50	123
BRWT	0.51880	0.40145	0.59013	-0.64635
	0.0001	0.0001	0.0001	0.0001
	123	123	50	123
AGE	-0.12787	-0.19688	-0.06800	0.05032
	0.1536	0.0271	0.6354	0.5804
	126	126	51	123
SIZE	0.28471	0.17769	0.07399	0.09514
	0.0012	0.0465	0.6059	0.2952
	126	126	51	123
POTRAD	0.14307	0.18056	0.08917	0.03255
	0.1100	0.0431	0.5338	0.7208
	126	126	51	123

Table B.3 (continued)

	BRWT	AGE	SIZE	POTRAD
DIFN	0.51880	-0.12787	0.28471	0.14307
	0.0001	0.1536	0.0012	0.1100
	123	126	126	126
LGINCR5	0.40145	-0.19688	0.17769	0.18056
	0.0001	0.0271	0.0465	0.0431
	123	126	126	126
INVSLA	0.59013	-0.06800	0.07399	0.08917
	0.0001	0.6354	0.6059	0.5338
	50	51	51	51
FOLBR	-0.64635	0.05032	0.09514	0.03255
	0.0001	0.5804	0.2952	0.7208
	123	123	123	123
BRWT	1.00000	-0.08347	0.07782	0.02070
	0.0	0.3587	0.3923	0.8203
	123	123	123	123
AGE	-0.08347	1.00000	0.17748	-0.03157
	0.3587	0.0	0.0468	0.7256
	123	126	126	126
SIZE	0.07782	0.17748	1.00000	0.00131
	0.3923	0.0468	0.0	0.9884
	123	126	126	126
POTRAD	0.02070	-0.03157	0.00131	1.00000
	0.8203	0.7256	0.9884	0.0
	123	126	126	126