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Multiple causes of seedling rarity in scrub plum, *Prunus geniculata* (Rosaceae), an endangered shrub of the Florida scrub[1]

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Conservation of an imperiled plant often requires an understanding of its reproductive ecology. Scrub plum (*Prunus geniculata*) is an endangered Florida shrub endemic to pyrogenic xeric uplands. Although plants are long-lived and may flower profusely, particularly after burning, fruit yield is sparse and seedlings are rare. We investigated potential causes of seedling rarity in scrub plum by studying its floral sex and breeding systems, fruit development, and germination ecology. We also developed a flow chart based on two time-since-fire scenarios to illustrate the cumulative impact of these factors on seedling recruitment. Breeding system experiments suggested partial gametophytic self-incompatibility in this functionally andromonoecious species, with inbreeding depression in self-compatible individuals. Predispersal seed predation, the major constraint on seedling recruitment, was lower in the first postburn season. In two field experiments, seed germination was <11% irrespective of treatment. These results indicate that seedling recruitment in scrub plum may be limited by multiple factors, including sexual reproductive failure, high predispersal seed predation, and low seed germination. The chance of a bisexual flower producing a seedling ranges from 5 in 1000 to 5 in 100 000, depending on the time since fire. Restoration of scrub plum's historical fire regime may mitigate some of these factors.

Key words: andromonoecy; endangered species; fruit maturation; inbreeding depression; Lake Wales Ridge; predispersal seed predation; *Prunus geniculata*; Rosaceae; seed germination.

Conservation of an imperiled plant species may require an understanding of several aspects of its reproductive ecology, including its sexual system, breeding system, pre- and postdispersal seed predation, and germination ecology. An integrated understanding of a species' reproductive ecology is particularly important when one or more of these factors may contribute to lack of seedling recruitment, thereby undermining the persistence of its populations.

In nonclonal species, lack of seedling recruitment inevitably results in population declines, even for a long-lived species (Stuefer et al., 2001). Low seedling recruitment may be a function of several nonmutually exclusive factors. Seed production may be limited by a scarcity of compatible mates (DeMauro, 1993; Weekley and Race, 2001), inadequate pollinator service (Bierzychudek, 1981; Kearns et al., 1998), resource limitation (Stephenson 1981), inbreeding (Husband and Schemske, 1996) or outbreeding (Waser, 1993) depression, or predispersal seed predation (Janzen, 1971; Crawley, 2000). Seed deposition into safe sites may be limited by inadequate seed dispersal (Willson and Traveset, 2000) or by postdispersal seed predation (Crawley, 2000; Auld and Denham, 2001). Seeds safely deposited into favorable microsites may fail to germinate due to the absence of germination cues (Bewley, 1997), and seed banks may decay over time due to additional predation or seed death (Thompson, 2000). Additionally, recruitment failure may be caused or exacerbated by anthropogenic alterations of biotic interactions (Wright and Duber, 2001) and abiotic conditions (Restrepo et al., 1999; Bruna, 2002).

Factors contributing to seedling recruitment failure may be particularly acute in rare species because of small population sizes (Menges, 1991; Brys et al., 2004), constraints imposed by the breeding system (DeMauro, 1993, Weekley and Race, 2001; Liu and Koptur, 2003), or habitat fragmentation (Wright and Duber, 2001; Bruna, 2002). Alteration of the natural disturbance regime of a species' habitat may also adversely affect its reproductive biology (Pickett and White, 1985). In particular, fire suppression may reduce flowering and fruit production (Bond and van Wilgen, 2007) and seedling recruitment (Keeley et al., 2005) in some pyrogenic species.

Scrub plum, *Prunus geniculata* Harper (Rosaceae), is a federally listed endangered shrub endemic to fire-maintained scrub and sandhill habitats on central Florida's Lake Wales Ridge (USFWS, 1999). About 85% of the xeric upland habitats on the Lake Wales Ridge have been lost to development or agriculture (Turner et al., 2006; Weekley et al., 2008), and the remaining habitat is fragmented and often fire-suppressed. Scrub plum individuals are long-lived and aggressively resprout following fire (Weekley and Menges, 2003; Menges et al., 2008). Mature plants often flower profusely, but fruit set is low in wild populations. Flowers appear morphologically perfect, but there is conspicuous variation in style length among flowers on a plant. In some flowers, pistils wither soon after flower opening, suggesting loss of female function. Seedlings are so rare in the wild that determining the causes of their rarity is the central focus of

the U.S. Fish and Wildlife Service's recovery plan (USFWS, 1999). In annual censuses of ~900 scrub plum plants from 2002 through 2008, Menges et al. (2008) recorded only two seedlings.

Most of the 250 species of *Prunus* are hermaphrodites (Wen et al., 2008), but other forms of gender expression have been documented, including andromonoecy (Tomlinson, 1980; Wolfe and Drapalik, 1999) and gynodioecy (Jordano, 1993). S-RNase-based gametophytic self-incompatibility (GSI; Franklin-Tong and Franklin, 2003) is characteristic of the genus (e.g., Hauck et al., 2002), but in some species self-compatible individuals or populations have also been documented (Dicenta and Garcia, 1993; Tao et al., 2000; Hauck et al., 2002; Sonneveld et al., 2005). Low fruit to flower ratios are common among flowering plants, including many *Prunus* species (Stephenson, 1981). Before our study, there had been no investigation of the breeding system of scrub plum, although Weekley (1997, unpublished manuscript) had reported andromonoecy from some populations.

Fire promotes increased flowering in scrub plum in the second postburn year (Menges et al., 2008). Flowering declines with time since fire, and older individuals flower sparsely or not at all. Fire also influences the quality of the microhabitat surrounding scrub plum plants, and thus potential seedling recruitment sites. For example, in fire-suppressed sites, the buildup of leaf litter and shade from neighboring shrubs may inhibit seedling establishment.

To investigate factors contributing to seedling rarity in scrub plum, we conducted a multiyear, integrated study of its floral sex and breeding systems and fruit and seed ecology. We had four main objectives: (1) to confirm the floral sex system and to document the spatial distribution of floral morphs and temporal changes in floral morph ratios, (2) to determine the breeding system and to compare fruit set in experimentally selfed and outcrossed flowers, (3) to quantify predispersal fruit loss and investigate the causes of premature abscission, and (4) to investigate the roles of postdispersal seed predation and microhabitat in limiting seed germination. To illustrate the cumulative impact of the breeding system, premature fruit loss, and low germination on seedling recruitment, we constructed a flow chart that integrated survival and loss of potential fruits from flowering to seedling recruitment based on two scenarios with different times since fire.

MATERIALS AND METHODS

Study species—Scrub plum is a multistemmed but nonclonal shrub to 2 m in height (USFWS, 1999). Within its pyrogenic xeric upland habitats, scrub plum is an edaphic generalist (Menges et al., 2007), occurring in longleaf pine–wiregrass [*Pinus palustris* Mill.–*Aristida stricta* Michx. var. *beyrichiana* (Trin. & Rupt.) D. B. Ward] sandhills, Florida rosemary scrub, and scrubby flatwoods (USFWS, 1999). Robust postburn plants produce a profusion of conspicuous flowers measuring ~1 cm in diameter. Scrub plum flowers are fragrant and attract a diversity of insects, including bees and butterflies. The fruit is a single-seeded, ovoid drupe 1–2.5 cm long. Flowering occurs in February–March and fruit set in April–May.

Study sites—We conducted field research on scrub plum at three sites on the Lake Wales Ridge: Archbold Biological Station, the Lake Wales Ridge National Wildlife Refuge/Carter Creek (hereafter, Carter Creek), and Bok Tower Gardens. The Archbold Reserve population comprises ~30 scrub plum plants in a semi-improved bahiagrass (*Paspalum notatum* Flüggé) cattle pasture. In contrast, the Carter Creek population consists of hundreds of plants occupying a 120-ha longleaf pine–wiregrass sandhill site. At Bok, we used a small wildland

population as well as plants in an ex situ collection. A second Archbold site, Red Hill, was used for a field germination experiment. The Carter Creek site was burned under prescription by the U.S. Fish and Wildlife Service in August 2001. However, scrub plum plants used in the 2002 and 2003 hand-pollination and fruit fate experiments (see below) were not burned.

Documenting the floral sex system (gender expression) in scrub plum—We identified floral sex morphs by dissecting flowers in the laboratory. On each flower, we measured the length of the pistil (overall), the ovary and the style; we also counted the number of stamens. We assigned each flower to one of three floral morphs based on style length. Flowers representing the three morphs were photographed using an Olympus SZX12 dissecting microscope and a 1-CCD digital video camera (Optronics, Goleta, California, USA). To document floral sex ratios in the field, we followed over 2400 flowers on 15 plants in two populations from bud-break to fruit initiation or abscission, noting the morphology of each developing flower in daily or more frequent surveys. We conducted field surveys in 2001 in the ex situ population at Bok Tower Gardens and in 2007 in the Archbold Reserve.

In 2001, we determined the spatial distribution of floral sex morphs within a plant by counting the number of morphs within each of three branch segments based on distance from the branch tip (<11.25, 11.25–24.75 and ≥25 cm). We marked two or more flowering branches on each of five plants in the Bok ex situ population. We subdivided branches into segments of approximately equal length and "mapped" the location of each bud by sketching the configuration of buds within each segment. Mapped branches were surveyed twice daily (to account for late-opening flowers) between 16 and 23 February 2001. In total, we scored 595 flowers over 8 d. All plants lost some buds due to herbivory or abortion, but overall 85% of the mapped buds opened (range 72–98%).

To document changes in the proportion of the three floral sex morphs over time, we counted the number of flowers belonging to each floral morph in daily surveys at Bok in 2001 and in the Archbold Reserve in 2007. At Bok, the survey coincided with the spatial distribution survey described above. In the Archbold Reserve, we selected one or more flowering branches on 10 plants. Each morning for 15 d, we recorded the floral morph of each newly opened flower and marked the flower with india ink to prevent double-counting. Between 28 January and 11 February 2007, we tallied and sexed 1828 flowers.

Determining the breeding system and comparing fruit set of selfed vs. outcrossed flowers—We conducted experimental hand pollinations to determine the breeding system of scrub plum and to compare fruit set in selfed vs. outcrossed flowers. In 2001 in the ex situ Bok population, we tested for agamospermy (production of viable fruits without fertilization), autogamy (spontaneous selfing), self-compatibility, and cross-compatibility. Application of this protocol resulted in four treatments requiring the bagging and/or emasculation of flowers to exclude unwanted pollen transfers, and an open-pollinated control (Table 1). For treatments requiring bagging of flowers, we used Delnet heat-sealed nonwoven pollination bags (Applied Extrusion Technologies, Middleton, Delaware, USA) designed to minimize alteration of the microenvironment. Of the 859 flower buds sequestered for the breeding system experiment, almost two-thirds were lost before opening due to abortion (38.2%) or herbivory (1.6%), or had vestigial pistils (23.3%); 317 flowers were included in the experiment.

In 2002, 2003, 2005, and 2007, we conducted additional hand pollinations in the Archbold Reserve, Bok, and Carter Creek populations to obtain a larger data set for fruit set in outcrossed vs. selfed flowers. The number of plants included in the hand-pollination experiments varied from five in 2001 to 10 or more in subsequent years. On each plant, depending on the number and accessibility of buds, we placed one to several pollination bags to exclude unwanted

TABLE 1. Experimental design of 2001 *Prunus geniculata* breeding system experiment at Bok Tower Gardens, Florida. The outcrossing (xenogamy) treatment involved crossing flowers from different plants; in the selfing treatment, pollen from each flower was applied to stigma of the same flower.

Treatment	Agamospermy	Autogamy	Selfing	Outcross	Control
Bagging	X	X	X	X	
Emasculation	X		X	X	
Hand pollination			X	X	
Number of flowers	68	54	81	50	64

pollinators. In all years, we used pollen from the same flower for self pollinations. For outcrosses (xenogamous crosses), we collected anthers from donor flowers that had been bagged prior to anthesis to prevent pollen contamination. Pollen transfer generally involved rubbing the anther from the donor onto the stigma of the recipient, although we occasionally used forceps to transfer pollen. We used both long-style and short-style bisexual morphs as pollen recipients. As in 2001, flower abortion, flower fragility, and the large number of male flowers in some treatment bags limited the number of hand pollinations.

In the 2001 breeding system study at Bok, we recorded the presence of ripe fruits, but did not closely follow fruit development. In all subsequent years, we followed developing fruits from initiation until they abscised or matured.

For the cross-compatibility experiments, we compared mean proportional fruit initiation and fruit maturation for selfed vs. outcrossed flowers using χ^2 tests (SPSS version 11.5; SPSS, 2002). To interpret our results, we also used a self-incompatibility index (SII) derived from Ramirez and Brito (1990), whereby SII = F_s/F_o, and F_s and F_o are the proportional fruit production of selfed and outcrossed flowers, respectively; SII values > 0.3 indicate self-compatibility. To explore differences in premature abscission rates between the selfing and outcrossing treatments, we calculated SII for both fruit initiation and fruit maturation.

Pollen tube inhibition—In plant species with S-RNAse-based gametophytic self-incompatibility (GSI), self crosses, or crosses between plants belonging to the same *S*-locus mating type, do not result in fertilization (except in cases where GSI has broken down). The incompatibility reaction usually takes place in the style, where pollen tube growth is arrested (Newbigin et al., 1993; Wheeler et al., 2001; Franklin-Tong and Franklin, 2003). Microscopic examination of hand-pollinated flowers can detect the site of pollen tube growth inhibition. If scrub plum has an S-RNAse-based GSI system, we expected to find pollen tube growth arrested in the styles of selfed flowers and of outcrossed flowers receiving pollen from plants belonging to the same *S*-locus mating type. In 2007, we hand-pollinated 40 scrub plum flowers (20 at Bok and 20 at Archbold Reserve) with either self pollen or pollen from a separate plant and collected them after 2 days. Flowers were preserved in 70% ethanol. We used epiflorescence microscopy to examine pollen tube growth in these flowers.

Quantifying predispersal fruit loss from premature abscission or predation—To quantify predispersal fruit loss and determine its causes, we conducted fruit fate experiments in the Carter Creek sandhill population in 2002, 2003, 2005 and 2007 (Table 2). In each year, we closely monitored flowering plants to identify branches where fruit initiation had occurred. On each fruiting shrub, we bagged some branches with developing fruits and marked other branches with labeling tape for an unbagged treatment; unbagged fruits were marked with a Sharpie pen to permit the recovery of abscised fruits. For the bagged treatment, we used Delnet pollination bags, as in the hand-pollination experiments. In most cases, each plant had both bagged and unbagged treatments.

We monitored fruits at 5–8-d intervals until all fruits had abscised. In each observation, we counted the number of surviving bagged and unbagged fruits on each plant, recorded any evidence of herbivory, and noted the date of apparent fruit maturation. To determine the cause of premature fruit drop, we collected abscised fruits for examination in the laboratory. We reared grubs obtained from predated fruits to identify the seed predator. In 2007, we also conducted searches around study plants for the plum curculio (*Conotrachelus nenuphar* Hebst., Curculionidae; Coleoptera), a weevil known to predate other species of plums.

We recognized five potential causes of premature fruit abscission: abortion, superficial damage, partial frugivory (fruits chewed into), weevil infestation, and damage/removal of bags by mammals or birds (bagged treatment only). Aborted fruits had no external signs of damage, while chewers and weevils left

telltale signs of their activities. Chewers generally consumed a quarter or more of the pulp surrounding the endocarp and ate the developing seed. Weevils left small exit holes in the fruit, and we often found grubs or frass in weevil-infested fruits.

We calculated the percentage of fruits lost in each category based on the total number of marked fruits in each of the two treatments. To compare maturation rates of bagged and unbagged fruits from the 2007 field experiment, we used *t* tests and survival analysis based on the life table procedure in SPSS version 11.5 (SPSS, 2002). This procedure computes median survival time for each treatment group and compares the survival distributions using the Wilcoxon (Gehan) statistic. To evaluate differences in the sources of premature abscission between the two treatments, we used χ^2 tests (SPSS version 11.5; SPSS, 2002). We restricted these analyses to the 2007 experiment because it had the largest sample size and the most detailed observations.

Field germination experiments—We investigated the germination ecology of scrub plum by conducting two year-long field experiments. The first experiment was installed on Red Hill at Archbold Biological Station in June 2006 and the second at Carter Creek in June 2007. Both experiments were designed to assess the effects of litter and caging (to reduce postdispersal seed predation) on the germination of intact scrub plum seeds. For both experiments, we collected seeds in April and May before sowing in June; we stored seeds in paper bags under ambient conditions.

The Red Hill experiment included three factors, each with two levels (shade/no shade, litter/no litter and caged/uncaged; five replicates, each replicate with 15 seeds; N = 600). For the shade treatment, we constructed shade houses consisting of 70% shade cloth attached to a 38 cm × 38 cm frame with legs 30 cm in length. The legs were driven into the sand to a depth of ~15 cm. For the litter treatment, we added oak–pine litter to a depth of ~5 cm. For the caged treatment, we attached 635-mm² square hardware cloth cages to the ground with 20 cm long nursery staples. Treatments were randomly assigned. We monitored the experiment weekly for seedling emergence and survival.

The Carter Creek experiment included two factors (litter/no litter and cage/uncaged) comprising four treatment combinations and eight randomly located replicates. Each treatment included 216 seeds (N = 864 seed), 27 seeds per replicate. For the no-litter treatments, we removed all litter from the area within which seeds were planted, while leaving litter in place for the litter treatment. For the caged treatment, we followed the same procedure as in the Archbold experiment. We monitored the experiment at 7–10-d intervals. In each visit, we also repaired damaged cages and removed any litter that had accumulated within the no-litter treatments. At the end of the Carter Creek experiment we retrieved ungerminated seeds by digging up the 30 cm × 25 cm sowing area.

To evaluate the effects of litter and caging on seed germination in the Carter Creek experiment, we performed a loglinear analysis using an algorithm written by Dr. Pedro Quintana-Ascencio (Department of Biology, University of Central Florida, Orlando, Florida, USA) in the program R (R Development Core Team, 2007). The loglinear procedure analyzes frequencies of observations in a contingency table using two or more explanatory variables.

RESULTS

Gender expression (floral sex morphs)—On the basis of field observations and dissections of flowers in the laboratory (N = 74), we recognized three floral sex morphs in scrub plum (Fig. 1). Morphs with styles less than 2.5 mm long (mean ± SD: 0.9 ± 0.36 mm) invariably had atrophied ovaries, indicating the loss of female function. Short-style morphs had styles averaging 3.5 ± 0.63 mm long, making them taller than the rim of the floral cup but shorter than the stamens. Long-style morphs had styles averaging 5.6 ± 0.46 mm, making them as tall as or slightly taller than the stamens. We classified flowers with vestigial pistils as males, and those with short- or long-style pistils as bisexuals. Both short- and long-style bisexuals had well-developed ovaries, usually with two ovules (however, mature fruits contain only a single seed). The mean number of stamens (mean ± SD: 29.7 ± 2.5 for males, 29.0 ± 2.4 for short-style bisexuals, and 29.8 ± 3.2 for long-style bisexuals) did not differ significantly among the three morphs (F = 0.769, df = 2, P =

TABLE 2. Experimental design of 2002, 2003, 2005 and 2007 *Prunus geniculata* fruit fate experiments at Carter Creek, Florida.

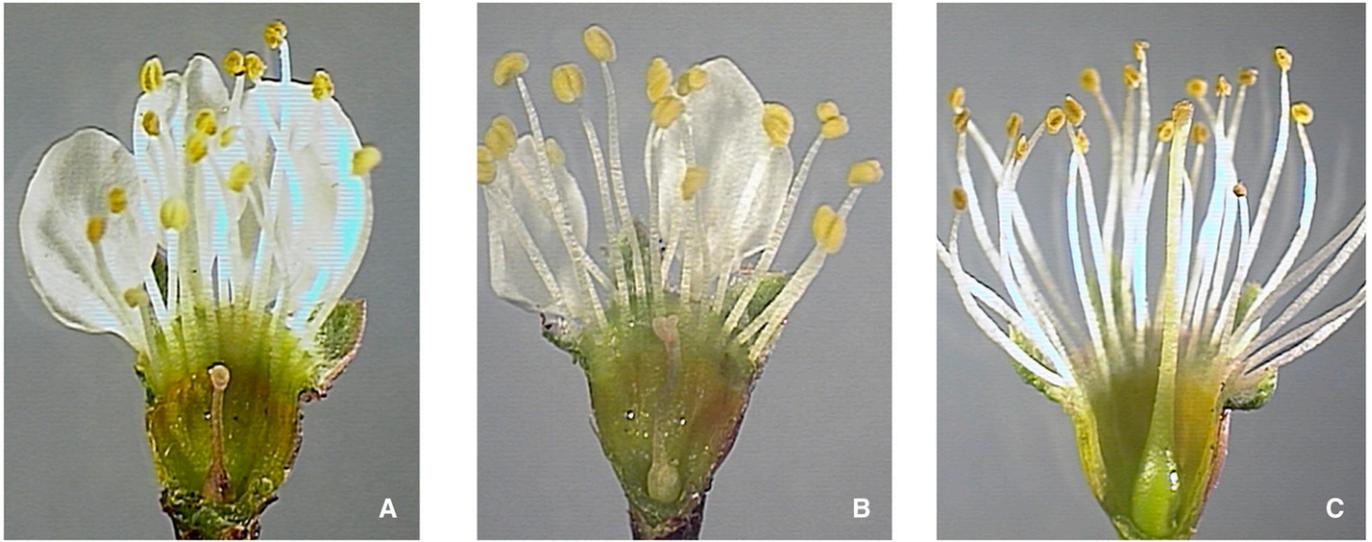| Year | No. plants | Unbagged | | Bagged | |
		No. branches	No. fruits	No. branches	No. fruits
2002	8	11	182	14	98
2003	7	9	66	9	65
2005	39	18	225	21	217
2007	23	57	706	61	648

Fig. 1.   Three *Prunus geniculata* floral sex morphs. (A) Male flower with vestigial pistil; (B) short-style bisexual flower; (C) long-style bisexual flower. Flowers were photographed with an Optronics digital video camera mounted on an Olympus SZX12 dissecting microscope at 7× magnification. Photos by J. Ash.

0.467) and the anthers of all morphs contained abundant supple pollen.

There was considerable variation in floral sex morph proportions among plants and over time in the field surveys. In the 2001 Bok survey, all five plants were dominated by bisexual morphs for the first few days of flowering, with few or no male flowers; by the end of the period, however, 40% of all open flowers were males, and two of five plants were dominated by male flowers. In the 2007 Archbold Reserve survey, 81.1% of 1828 surveyed flowers were males, 11.0% short-style bisexuals, and 7.9% long-style bisexuals. The cumulative proportion of males varied from 48.5 to 100% among the 10 study plants in the Archbold Reserve, and only four plants had more than 10% long-style bisexual flowers (Fig. 2A). During the 8-d period when 96% of flowers opened, the mean proportion of male flowers among the 10 plants increased from 41.7 to 83.7% (Fig. 2B), while the mean proportion of short-style and long-style bisexuals decreased from 20.6 to 2.3% and from 26.5 to 13.9%, respectively. Thus, in both the Bok and Archbold surveys, the proportion of flowers capable of setting fruit decreased both day to day (i.e., among newly opened flowers) and cumulatively (i.e., among all flowers) over the course of the flowering season.

The proportion of floral sex morphs also varied spatially within a plant, as measured by distance from the tip of the flowering branch. In the 2001 Bok survey, sex morph proportions differed significantly depending on a flower's location along the branch ($\chi^2 = 27.456$, df = 4, $P < 0.001$). Long-style bisexuals predominated proximally (≥25cm from branch tip), short-style bisexuals predominated distally (<11.25cm from branch tip), and male flowers were found in similar proportions along the branch (Fig. 3). Thus, flowers capable of setting fruits occurred along the entire length of the branch and were intermixed with male flowers.

Breeding system and selfing vs. outcrossing experiments—In the 2001 breeding system experiment, we obtained no fruits from the agamospermy, autogamy, or self-compatibil-
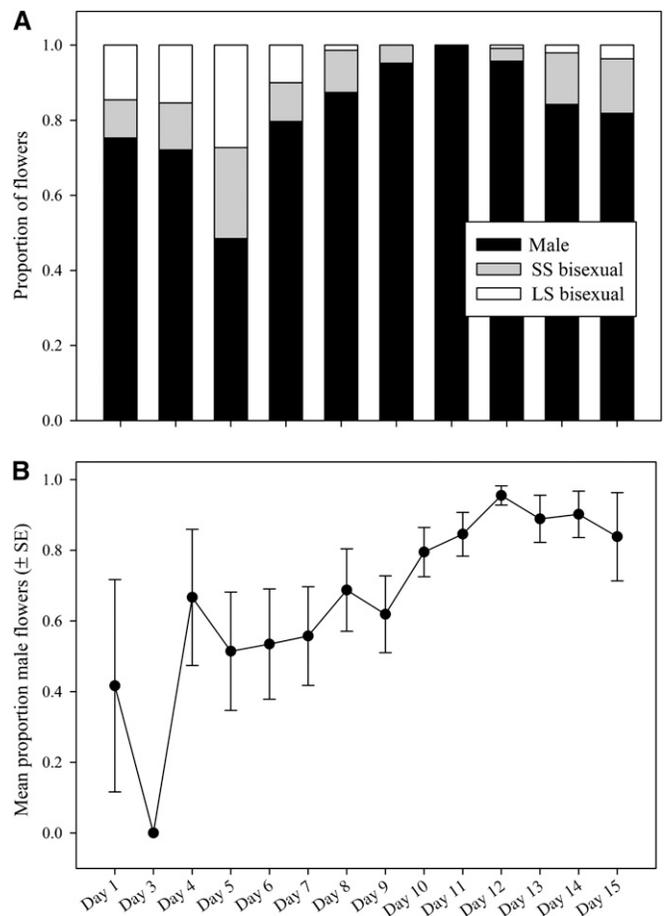

Fig. 2.   (A) Cumulative proportion of *Prunus geniculata* flowers in each of three floral sex morphs on 10 plants at Archbold Reserve (2007); (B) mean proportion of male flowers (±SE) over 15-d flowering season at Archbold Reserve based on the same 10 plants as in 2A. SS = short-style; LS = long-style.

ity treatments. Hand-pollinated outcrosses yielded a significantly greater percentage of mature fruits than open-pollinated controls ($N = 50, 64$; 18% vs. 4.7%; $\chi^2 = 5.282$, df = 1, $P = 0.022$). These results suggested that scrub plum is self-incompatible and perhaps pollinator-limited.

In the five subsequent selfing vs. outcrossing experiments, percentage fruit initiation and percentage fruit maturation often varied widely among sites and years (Table 3, Fig. 4A, b). For the 3 years in which percentage fruit initiation could be analyzed, self pollinations and outcrosses initiated statistically equal percentages of fruits, but selfed fruits suffered higher rates of abortion (Table 3; 2002 at Carter Creek, 2005 at Archbold Reserve, 2007 at Bok). Fruits were initiated in 3 of the 4 years for both treatments (Fig. 4A; 2007 was the exception), with initiation rates >45% for both selfs and outcrosses in the wild population at Carter Creek in 2002. At Carter Creek, a few self pollinations resulted in mature fruits in 2002 and 2003 (Fig. 4B), but percentage fruit maturation was only about 10% the maturation rate of outcrossed fruits. At Bok in 2007, as in 2001, no selfed flowers produced mature fruits. In the Archbold Reserve, no initiated fruits matured in 2005, and in 2007 no fruits were initiated in either treatment.

The self-incompatibility index (SII) generally indicated self-compatibility when based on percentage fruit initiation (Table 3; 3 of 3 years), but self-incompatibility when based on percentage fruit maturation (Table 3; 4 of 4 years). These results suggest that scrub plum is weakly self-compatible, but that fruits resulting from self crosses have high levels of abortion due to inbreeding depression.

In 2 of 3 years, percentage fruit initiation did not differ significantly between hand-pollinated outcrosses and open-pollinated controls; in the third year (2005), fruit initiation in the controls was four times greater than in the outcrosses (Table 3). Thus, for these years and populations, fruit initiation was not
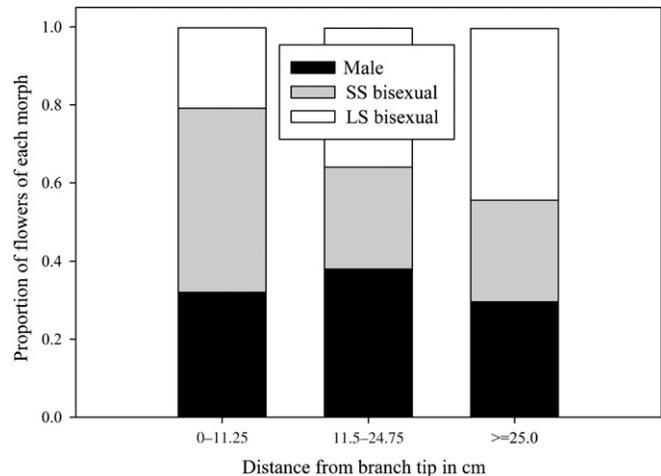

Fig. 3. Spatial distribution of three *Prunus geniculata* floral sex morphs as measured by distance from the tip of flowering branch. Proportions are based on a cumulative total of 476 flowers on 29 flowering branches on five plants. SS = short-style; LS = long-style.

limited by pollinator service. Fruit maturation rates did not differ significantly between outcrosses and controls in 2 of the 3 years for which data were available (Table 3).

Pollen tube inhibition—In the pollen tube inhibition study, 85% of selfed and outcrossed flowers from Bok and the Archbold Reserve had germinated pollen (Table 4). In 89.9% of self crosses, pollen tube growth was arrested in the style, as expected for a species with an S-RNase-based gametophytic self-incompatibility system, and pollen tubes only reached the ovary in outcrossed flowers. At Bok, 72.7% of the outcrossed flowers had pollen tube

TABLE 3. Summary of experimental results of the *Prunus geniculata* breeding system by year and site, showing rates of fruit initiation and maturation for selfed, outcrossed, and open (control) pollinations. Curly brackets indicate statistical comparisons between treatments based on χ^2 tests with df = 1. SII = F_s/F_o, where F_s and F_o are the proportional fruit yield of selfed and outcrossed flowers, respectively. Values >0.3 indicate self-compatibility. SII_I is based on fruit initiation; SII_M is based on fruit maturation. Bok = Bok Tower Gardens, CCS = Carter Creek, ABS = Archbold Reserve. NA = data not available.

Year, Site (no. plants)	Treatment	No. flowers	No. fruits initiated (%)	χ^2 tests for fruit initiation	No. fruits matured (%)	χ^2 tests for fruit maturation	SII
2001, Bok (5)	Selfed	81	NA	NA	0 (0)	} NA	SII_I = NA
	Outcrossed	50	NA		9 (18.0)	} $\chi^2 = 5.282$, $P = 0.022$ [a]	SII_M = 0
	Control	64	NA		3 (4.7)		
2002, CCS (12)	Selfed	185	92 (49.7)	} $\chi^2 = 0.377$, $P = 0.539$	3 (1.6)	} $\chi^2 = 49.588$, $P < 0.001$	SII_I = 1.07
	Outcrossed	170	79 (46.5)	} $\chi^2 = 0.543$, $P = 0.461$	47 (27.7)	} $\chi^2 = 3.204$, $P = 0.073$	SII_M = 0.05
	Control	47	19 (40.0)		7 (14.9)		
2003, CCS (13)	Selfed	NA	96	NA	4 (NA)	NA	SII_I = NA
	Outcrossed	NA	99		36 (NA)		SII_M = 0.12
	Control	NA	29		0		
2005, ABS (9)	Selfed	1656	9 (5.5)	} $\chi^2 = 0.052$, $P = 0.820$	0	} No fruits matured	SII_I = 1.12
	Outcrossed	203	10 (4.9)	} $\chi^2 = 23.724$, $P < 0.001$	0	} No fruits matured	SII_M = NA
	Control	107	25 (23.4)		0		
2007, Bok (6)	Selfed	103	26 (25.2)	} $\chi^2 = 1.920$, $P = 0.160$	0	} NA	SII_I = 0.73
	Outcrossed	84	29 (34.5)	} $\chi^2 = 2.830$, $P = 0.093$	15 (17.9)	} $\chi^2 = 3.604$, $P = 0.058$	SII_M = 0
	Control	141	34 (24.1)		13 (9.2)		
2007, ABS (10)	Selfed	50	0	No initiation	0	No initiation	Pollinations
	Outcrossed	80	0		0		all failed
	Control	204	0		0		

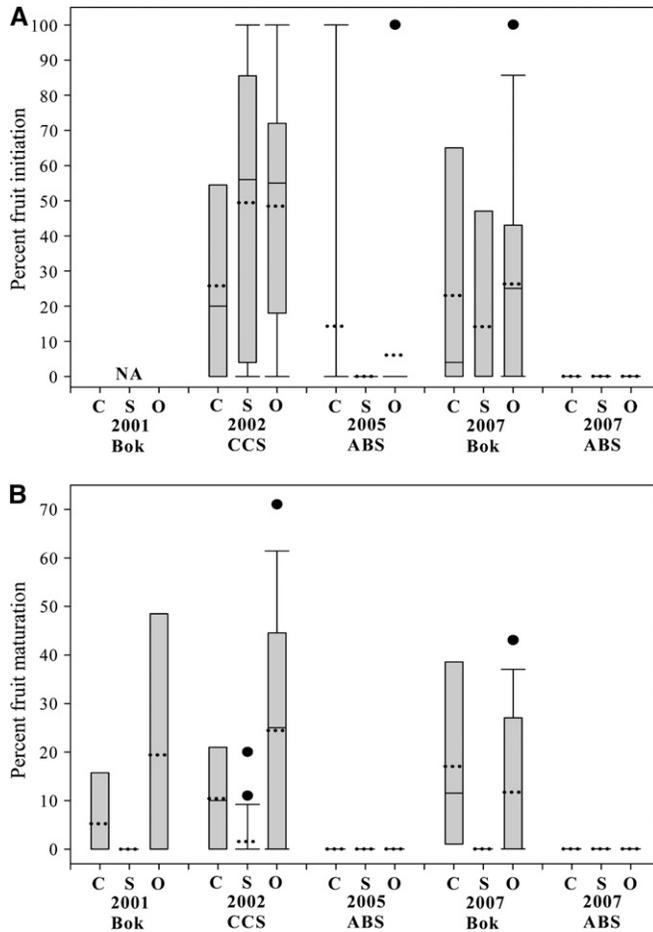[a] Calculated as percentage of flowers crossed.

Fig. 4.   Boxplots showing results of *Prunus geniculata* cross-compatibility experiments with hand-pollination self-crosses (S), outcrosses (O) and open-pollinated controls (C). (A) Fruit initiation and (B) fruit maturation. The lower and upper lines of shaded bars represent the 25th and 75th percentiles, respectively; the solid middle line represents the median and the dotted line represents the mean. The lower and upper "whiskers" show the largest and smallest values that are not outliers. The circles are outliers (1.5 box lengths from 25th and 75th percentiles). Where no shaded bar is present, the median is zero. The 2003 Carter Creek experiment is not shown because data are not available on number of flowers hand-pollinated.

growth into the ovary. In contrast, none of the outcrossed flowers from the Archbold Reserve had pollen tube growth into the ovary, suggesting that outcrossed plants in the Archbold Reserve population all belonged to the same *S*-locus mating type and that GSI had not broken down in these individuals.

Fruit fate—In the fruit fate experiments conducted in 2002, 2003, and 2005, bagged branches consistently matured a higher proportion of fruits than unbagged branches (Fig. 5), with no unbagged fruits maturing in 2 of the 3 years. For both treatments, fruit maturation was higher in the first year postburn (2002) than in any subsequent year surveyed. In the 1 year in which both bagged and unbagged fruits matured (2002), the bagged treatment yielded significantly more fruits (34.7% vs. 15.4%: $\chi^2 = 13.8$, df = 1, $P < 0.0001$). In these 3 years, we retrieved 418 abscissed fruits; 52.4% of retrieved fruits showed signs of insect predation. In all years, unbagged fruits had a higher percentage of predated fruits than bagged fruits.

Table 4.   Results of pollen tube (PT) growth observations from hand-pollinated selfed and outcrossed *Prunus geniculata* flowers on the Archbold Reserve and at Bok Tower Gardens. PTs = pollen tubes.

Location	Cross	No. flowers	No. flowers with germinated pollen (%)	No. PTs arrested in style (%)	No. PTs in ovary (% fertilization)
Archbold	Self	11	11 (100)	9 (77.8)	0
	Out	9	5 (55.6)	1 (20.0)	0
Bok	Self	9	7 (77.8)	7 (100)	0
	Out	11	11 (100)	3 (27.3)	8 (72.7)

In the 2007 fruit fate experiment (Fig. 5), we obtained 97 mature fruits from the bagged treatment (15.0% maturation rate) and only one fruit from the unbagged treatment (0.14% maturation rate), a difference of two orders of magnitude. Because it is difficult to pinpoint the exact date of fruit maturation, counts of mature fruits may either under- or overestimate maturation percentages. However, the difference in survival between the two treatments developed well before fruit maturation. Survival analysis revealed a significant difference between the survival curves of bagged and unbagged fruits (Fig. 6), with bagged fruits surviving longer than unbagged fruits (Wilcoxon [Gehan] statistic = 69.044, df = 1, $P < 0.0001$). The curves diverged sharply near the end of March and by mid-April, the earliest period for which we recorded maturation, there was a significant difference in the percentage of surviving fruits in the two treatments ($t = 4.9$, df = 36.9, $P < 0.0001$).

The causes of premature abscission in the 2007 experiment differed significantly between the bagged and unbagged treatments ($\chi^2 = 189.9$, df = 3, $P < 0.0001$; damage to bags not included in χ^2 test). Most abscission in the bagged treatment was due to abortion or superficial damage, while in the unbagged treatment most abscission was due to chewers or weevils (Table 5). Minor nicks or scratches on some abscised fruits seemed insufficient to cause abscission, and it seems likely that these fruits should be added to the aborted category. With the inclusion of these fruits, abscission due to abortion accounted for at least 42.2% of marked fruits in the bagged treatment vs. 25.2% in the unbagged treatment. Abscission due to insect damage accounted for 6.3% of fruit loss in the bagged treatment vs. 39.5% in the unbagged treatment. We saw no evidence that bagging increased abortion rates, but suspect that many fruits that would otherwise have aborted were also attacked by chewers or weevils. Fruit loss due to the destruction of bags used in the bagging treatment (probably caused by birds or mammals attracted to the bags) was an artifact of the treatment itself.

The proportion of abscised fruits in each category differed over time for the two treatments (Fig. 7). We recognized three periods: the abortion/chewer period (22 February–26 March), the weevil period (27 March–13 April), and the fruit maturation period (13 April–5 May). In the abortion/chewer period (Fig. 8A), 80.9% of bagged fruits abscised due to abortion (including fruits with superficial damage), while 52.0% of unbagged fruits were lost to chewers. In the weevil period (Fig. 8B), abortion continued to play a large role in fruit loss in the bagged treatment (43.2%), but weevils accounted for 15.3% of abscised fruits within bagged treatments, indicating that weevils oviposited on young fruits prior to bagging. For unbagged fruits during the weevil period, weevils accounted for 22.5% of fruit loss and chewers were of minor importance. In the fruit maturation period (Fig. 8C), the percentage of bags ripped off plants increased dramatically in the bagged treatment (82.6%). For the unbagged treatment during fruit maturation, we were not able
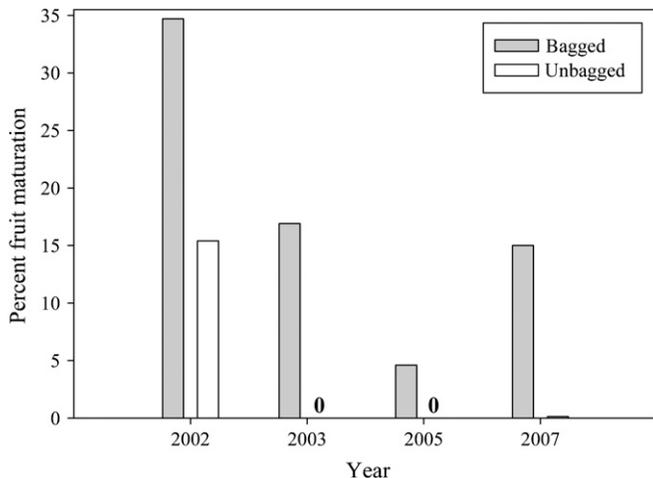
Fig. 5. Results of Carter Creek *Prunus geniculata* fruit fate experiments for 4 years showing total percentage fruit maturation for bagged and unbagged fruits.

to recover enough fruits to make meaningful calculations. Over the three periods, 91.2% of abscised fruits in the bagged treatment and 64.9% in the unbagged treatment were accounted for.

A new host record for the plum curculio—In 2007, we captured nine adult weevils on or near fruiting scrub plum plants and reared 28 adults from grubs obtained from infested fruits. The weevil was identified by Dr. Mark Deyrup (Entomology Laboratory, Archbold Biological Station, Lake Placid, Florida, USA) as the plum curculio, *Conotrachelus nenuphar* Hebst. (Curculionidae: Coleoptera).

Germination ecology—Irrespective of treatment, seed germination was low in the two field experiments. In the Red Hill experiment, overall germination was 1.2% and five of the eight treatments had no germination at all, precluding further analysis. In the Carter Creek experiment, overall germination was
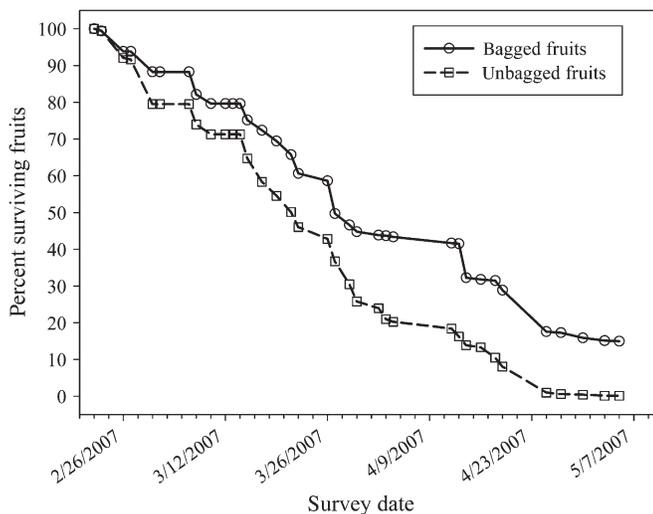

Fig. 6. Survival curves for bagged and unbagged *Prunus geniculata* fruits in 2007 Carter Creek fruit fate experiment.

6.7%, was higher with either litter or caging, and was highest in the caged/litter treatment (Fig. 8). Loglinear analysis of the Carter Creek experiment revealed that germination was significantly affected by the two treatments ($G^2 = 8.78$, df = 3, $P = 0.0324$).

Altogether we retrieved 51.5% of the ungerminated seeds from the Carter Creek experiment. The percentage of recovered seeds was almost twice as high from the caged treatment as from the uncaged treatment (67.5% vs. 35.9%) and the difference was significant ($\chi^2 = 80.359$, df = 1, $P < 0.0001$). However, there was no significant difference between the two treatments in the percentage of recovered seeds that were undamaged (uncaged = 78.2%, caged = 80.2%; $\chi^2 = 0.231$, df = 1, $P < 0.630$).

DISCUSSION

Three of the four factors investigated in this study may contribute to low seedling recruitment in scrub plum. Among these factors, premature fruit loss, due to predation or abortion, imposed the most significant constraint on potential seedling recruitment. Although we recorded fruit initiation rates as high as 40% in open-pollinated flowers, the percentage of unprotected fruits that survived to maturity was never greater than 15%, and in 3 of 4 years it was less than 1%. Low seed germination is also a likely contributor to seedling recruitment failure because field germination rates never exceeded 11% irrespective of treatment. The role of scrub plum's partial gametophytic self-incompatibility system is more difficult to assess, but it may contribute to low seed production under some circumstances. Scrub plum's andromonoecious floral sex system played no role in limiting seedling recruitment.

Floral sex system—Scrub plum is functionally andromonoecious: bisexual flowers and male flowers containing vestigial pistils occur on the same plant. Andromonoecy, known from about 2% of angiosperms (Richards, 1997), has been documented in two other *Prunus* species, West Indian cherry (*P. myrtifolia*; Tomlinson, 1980) and Carolina laurel cherry (*P. caroliniana*; Wolfe and Drapalik, 1999). Scrub plum differs from these congeners in details of flower morphology and spatial arrangement. The stylar dimorphism of scrub plum's bisexual flowers is apparently unique among andromonoecious species, but the functional significance of the two bisexual variants is unknown.

Andromonoecy is usually interpreted as an adaptation to optimize investment in male and female function by diverting resources from the production of bisexual flowers in favor of less costly male flowers (Bertin, 1982; Vallejo-Marin and Rausher, 2007a). As expected under this hypothesis, numerous studies have shown that sex expression is variable among andromonoecious individuals and populations (e.g., May and Spears, 1988; Diggle, 1994; Miller and Diggle, 2003) and that the proportion of male flowers increases over time (e.g., Primack and Lloyd, 1980; May and Spears, 1988; Miller and Diggle, 2003). Scrub plum conforms to these expectations by displaying high variability in sex expression and a pronounced temporal increase in the proportion of male flowers. Because fruit production is resource limited (Primack and Lloyd, 1980), the increase in the proportion of male flowers may enhance female fitness by freeing resources for the production of additional fruits (Diggle, 1993; Vallejo-Marin and Rausher, 2007b). In scrub plum, most

TABLE 5. Sources of *Prunus geniculata* fruit loss in 2007 Carter Creek fruit fate experiment, calculated as percentage of marked fruits (N = 648 for bagged treatment, 706 for unbagged treatment) that were lost prior to maturation. NA = not available.

Source of loss	Bagged		Unbagged	
	No. fruits	% Fruits	No. fruits	% Fruits
Abortion	185	28.5	120	17.0
Superficial damage	89	13.7	58	8.2
Chewer	18	2.8	226	32.0
Weevil	23	3.5	53	7.5
Bagged ripped off	179	27.6	NA	NA
Fate unknown	57	8.8	248	35.1
Total loss	551	85.0	705	99.9

bisexual flowers failed to initiate fruits even when experimentally outcrossed. Thus, scarcity of bisexual flowers was not a constraint on fruit production.

Breeding system—The effect of scrub plum's gametophytic self-incompatibility (GSI) system on seedling recruitment is complicated and likely depends on environmental conditions. Data from experimental hand pollinations and observations on pollen tube growth inhibition support the hypothesis that scrub plum has partial GSI, whereby the incompatibility reaction may be attenuated under some circumstances. Fruit initiation rates did not differ significantly between selfed and outcrossed flowers, but outcrossed flowers had significantly higher rates of maturation. Correspondingly, the self-incompatibility index (SII) consistently identified scrub plum as self-compatible when based on fruit initiation rates, but self-incompatible when based on fruit maturation rates. Although there is no obvious mechanism for the unmediated transfer of pollen within a flower, geitonogamy is probably common as floral visitors move among flowers on a plant.

The lack of fruit initiation from either selfed or outcrossed hand pollinations in the 2007 Archbold Reserve experiment suggests that these plants belonged to the same self-incompatible *S*-locus mating type. Pollen tube growth inhibition in the styles of most selfed flowers is also consistent with GSI. However, the development of mature fruits in some selfed flowers is consistent with partial or incomplete GSI (Becarra and Lloyd, 1992; Stephenson et al., 2000), whereby the self-incompatibility reaction breaks down in some populations or environments (Good-Avila et al., 2001; Travers et al., 2004) or as flowers age (Vogler et al., 1998).

Where mates or pollinators are scarce, attenuation of self-incompatibility may provide reproductive assurance, but only in the absence of pollen or seed discounting or inbreeding depression (Lloyd, 1980, 1992; Barrett and Harder, 1996; Holsinger, 1996). For example, if self-incompatibility in scrub plum is relaxed as flowers age, then spontaneous delayed selfing (sensu Lloyd, 1992; Barrett and Harder, 1996) would not co-opt pollen or ovules that might otherwise be used in outcrossing and could provide reproductive assurance. However, spontaneous selfing (autonomous autogamy) has not been demonstrated in scrub plum. Insect-facilitated selfing and geitonogamy usually result in both pollen and seed discounting (Lloyd, 1992; Holsinger, 1996; Herlihy and Eckert, 2002) and thus provide no reproductive assurance. In addition, most scrub plum fruits initiated in selfed flowers failed to mature. Thus, even in the absence of pollen or seed discounting, high rates of fruit loss in
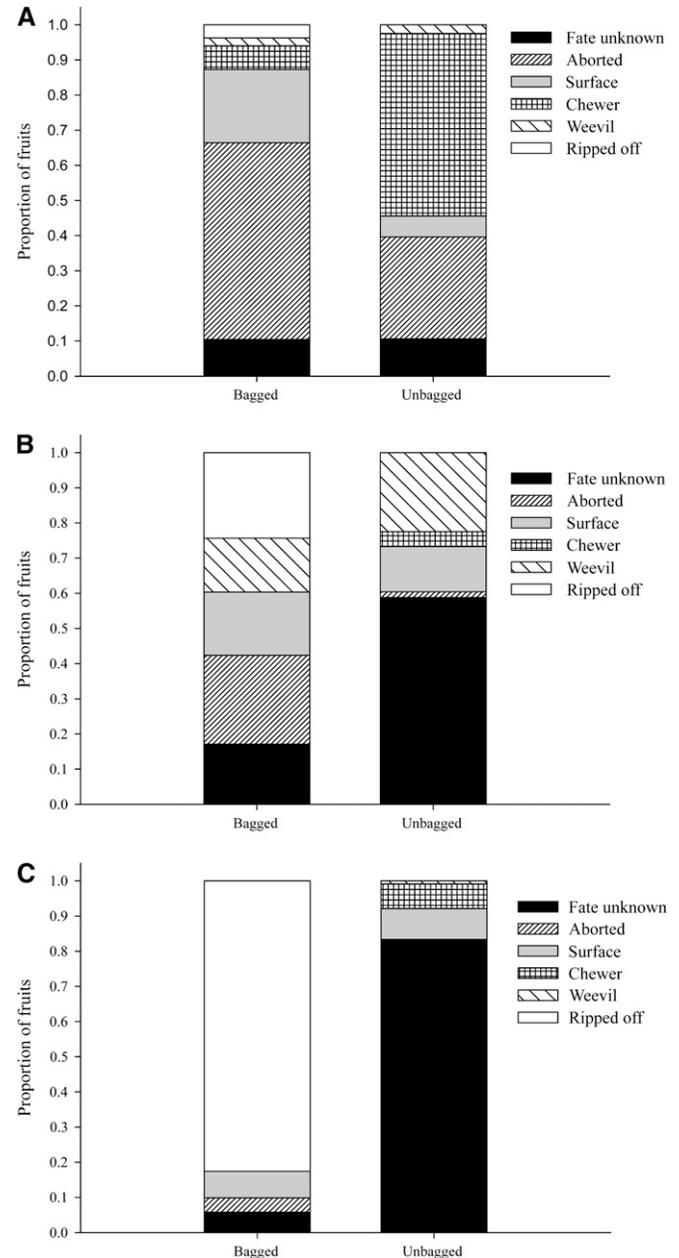

Fig. 7. Putative causes of fruit loss for bagged and unbagged *Prunus geniculata* fruits in three periods of fruit development: (A) abortion/chewer period; (B) weevil period; (C) fruit maturation period. For the unbagged treatment, only 19 fruits could be accounted for in the final period. "Surface" = superficial damage to fruit.

selfed flowers due to inbreeding depression (Holsinger, 1991, 1996; Barrett and Harder, 1996; Herlihy and Eckert, 2002) would prevent a significant role for reproductive assurance in scrub plum.

Premature fruit loss—In our study, premature fruit loss constituted the major constraint on potential seedling recruitment in scrub plum: in 3 of 4 years at Carter Creek, fewer than 20% of unprotected fruits in the fruit fate experiment reached maturity.
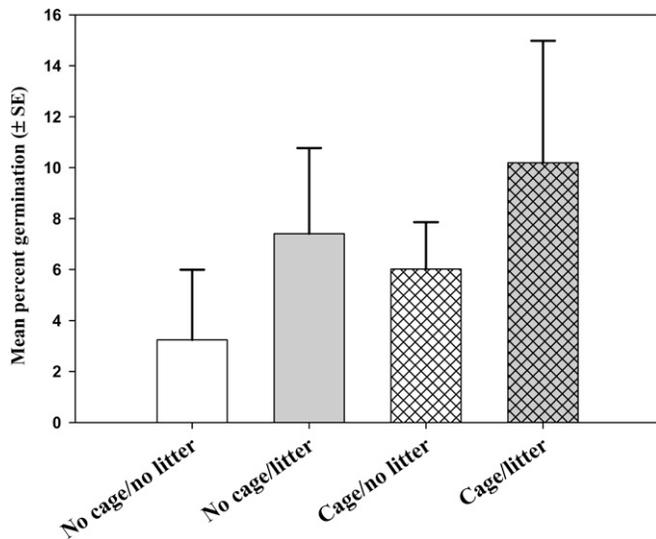
Fig. 8. Mean percentage germination (±SE) *Prunus geniculata* seeds in 2007 germination experiment at Carter Creek, showing four treatment combinations.

Interestingly, the year with the highest percentage maturation for both bagged and unbagged fruits was 2002, the year following a prescribed burn. By reducing frugivore and seed predator populations, fire may increase the percentage of scrub plum fruits that survive to maturity.

The major causes of fruit loss were abortion and predispersal fruit/seed predation. Abortion was more frequently recorded in the bagged than in the unbagged treatment; the opposite was true for fruit/seed predation. The apparently lower rate of abortion in the unbagged treatment was probably due to predation of unprotected fruits that would otherwise have aborted. The mesh pollination bags used in the bagging treatment do not alter the microenvironment of bagged fruit in any way likely to cause abortion.

Premature fruit abscission depresses fruit to flower ratios and both topics have been widely investigated (e.g., Stephenson, 1981; Sutherland and Delph, 1984; Sutherland, 1986a, b). Stephenson (1981) demonstrated that resource limitation often plays a significant role in reducing fruit yield. Limiting resources (including light, water, and mineral nutrients) may contribute to fruit loss in scrub plum, but it cannot account for the notable difference in maturation between selfed and outcrossed fruits, due to inbreeding depression.

In the scrub plum fruit fate experiments, the two major predators were the plum curculio and an unidentified frugivore. The plum curculio is a seed-predating weevil well known as an agricultural pest on the fruits of several species, including peaches, plums, and apples (Jenkins et al., 2006). Our study provides the first host record of the plum curculio on scrub plum. Reported rates of damage from the plum curculio are as high as 85% in some apple orchards (Vincent et al., 1999). The presence of plum curculio grubs in bagged fruits indicates that females were able to oviposit on small developing fruits prior to their being bagged. The unidentified frugivore was most likely an orthopteran (M. Deyrup, Archbold Biological Station, personal communication). Typically, young fruits were chewed into, and the developing seed was consumed. Chewer damage declined as fruits matured, perhaps because the seed became less accessible as the endocarp hardened.

The plum curculio is a native weevil, and it is likely that the scrub plum chewer is native as well. However, the absence of the frequent fires historically characteristic of Florida sandhill habitats may result in high populations of both predators. Fruit maturation rates in unbagged scrub plum fruits was at least two orders of magnitude greater after prescribed burning than in other years. The re-establishment of natural fire return intervals in scrub plum's sandhill habitats may result in reduced predator populations and less devastation to scrub plum fruits. Snapp et al. (1922) recommended the use of fire to reduce plum curculio populations in wooded areas near plum orchards.

Germination ecology—The second most important limitation on seedling recruitment found in this study was low seed germination. Germination percentages were less than 11% in the two experiments, irrespective of treatment. The highest germination percentage occurred in the cage + litter treatment at Carter Creek. Litter may promote germination by retaining moisture longer than sandy microsites devoid of litter. Although frequent burning of scrub plum's sandhill habitat would reduce both litter cover and depth, these effects are short-lived (Menges et al., 2008). The higher percentage of ungerminated seeds retrieved from the caged treatments also suggests that postdispersal removal/consumption of seeds by vertebrates may contribute to low germination rates.

Integrating multiple sources of seedling recruitment failure—By integrating data from the 2002 hand-pollination and fruit fate experiments and the 2007 fruit fate and seed germination experiments, we can project two scenarios to estimate cumulative survival from (bisexual) flowering to seedling emergence (Fig. 9). The two scenarios provide contrasting estimates of seedling recruitment under differing time-since-fire conditions. Both scenarios begin with the 40% fruit initiation rate for open-pollinated flowers from 2002, the first flowering season following the 2001 prescribed fire. This rate is the highest fruit initiation obtained in open-pollinated flowers in any of the hand-pollination experiments, and thus represents the most favorable starting point for the two scenarios that follow. In scenario 1, based on fruit maturation in the 2002 hand-pollination experiment (first postburn flowering season), 14.9% of initiated fruits survived to maturity. This initiation percentage is consistent with the 2002 fruit fate experiment, where an equal percentage (15.0%) of unbagged fruits survived to maturity, the highest level obtained in any of the four fruit fate experiments. In scenario 2, based on the 2007 fruit fate experiment (sixth postburn flowering season), fruit maturation was two orders of magnitude less (0.14%) than in the 2002 hand-pollination and fruit fate experiments, with most fruit loss attributable to predispersal seed predation. Further losses in both scenarios were due to seed germination failure, based on the 2007 germination experiment. We break down this loss by litter vs. no litter microsites. Survival in microsites with litter was nearly twice as high as for microsites without litter (8.8% vs. 4.6%). The cumulative survival from bisexual flowers through germinated seedlings varied by four orders of magnitude, with a high of 5 in 1000 surviving in scenario 1 seeds in microsites with litter to a low of 5 in 100 000 for scenario 2 seeds in microsites with no litter (bottom of Fig. 9).

Conclusion—The major contributing sources to seedling recruitment failure demonstrated by our research (low rates of fruit maturation, high seed predation, poor seed germination) may reflect human changes (habitat fragmentation, fire suppression)
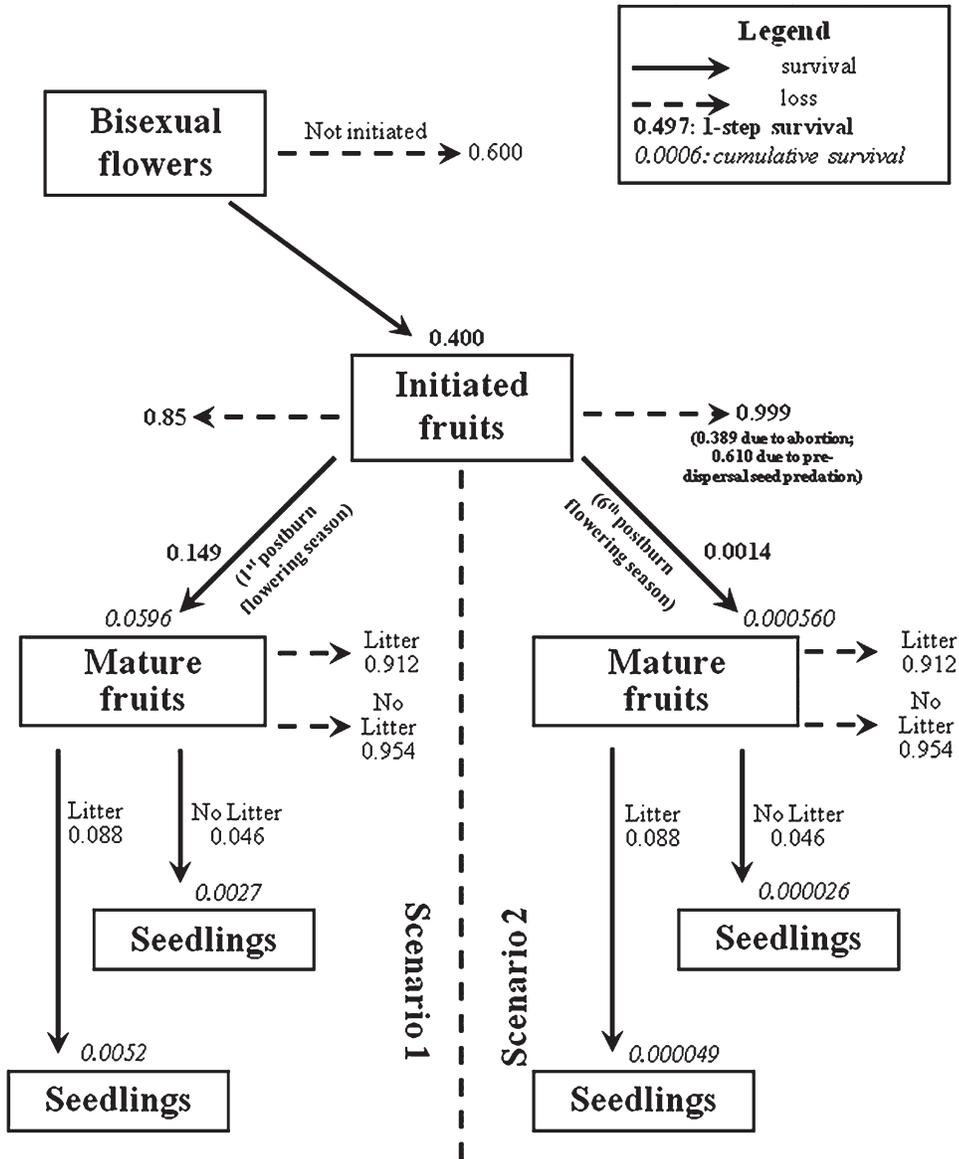
Fig. 9. Flow chart summarizing survival and loss data on fruit initiation, fruit maturation, and seed germination, based on data from the Carter Creek wild *Prunus geniculata* population (see text for more details on data sources). Boldface numbers represent the survival rate at each stage in fruit development; for example, the numbers next to the arrows in the transition from initiated fruits to mature fruits are the proportions of developing fruits not lost to abortion or predispersal seed predators in the two scenarios. Italicized numbers are cumulative survival; for example, the numbers alongside the arrowheads just above the mature fruits boxes are the product of the proportion of fruits initiated and the proportion of fruits surviving to maturity (0.400 times 0.149 in scenario 1 and 0.400 times 0.0014 in scenario 2). This product is the proportion of bisexual flowers that survive to become mature fruits. The fruit maturation rate in scenario 1 is based on open-pollinated flowers/fruits in the 2002 hand-pollination experiment (maturation rate in the 2002 fruit fate experiment was almost exactly the same); in 2002 we did not determine source of fruit loss. The fruit maturation rate in scenario 2 is based on flowers/fruits included in the 2007 fruit fate experiment, for which we provide data on sources of fruit loss. (Figure based on Fig. 2 in Campbell and Clarke, 2006).

in scrub plum's landscape. About 85% of scrub plum's original habitat has been lost to development (Turner et al., 2006; Weekley et al., 2008), and many remaining populations are small and isolated. Cox et al. (2004) found that 61% of scrub plum populations on protected sites comprised fewer than 10 plants. These tiny populations may suffer substantial inbreeding depression. In addition, remaining populations are embedded in an increasingly urbanized matrix that poses a significant challenge to the application of prescribed fire (Duncan and Schmalzer, 2004).

The combination of decreased flowering and low seed survival associated with fire suppression may account for a substantial portion of seedling rarity in scrub plum. Flowering increases in the second year postburn and declines thereafter (Menges et al., 2008). In addition, fire may increase the production of viable fruits by reducing populations of scrub plum's fruit/seed predators. Studies in other ecosystems have also found that burning may reduce seed predation (e.g., Vickery, 2002). Habitat fragmentation alone may increase a plant's susceptibility to seed predators (Garcia and Chacoff, 2007)

and other herbivores (Alverson et al., 1988; Roland, 1993; Roland and Taylor, 1997). The combined effects of habitat fragmentation and the absence of fire in scrub plum populations may result in the persistent loss of potentially viable seeds and contribute to a slow but inexorable decline in population sizes.

Scrub plum plants have high survival rates (>97% annually in the large Carter Creek population between 2001 and 2008; Menges et al., 2008) and are therefore likely long-lived. Although the longevity of scrub plum individuals and the apparent stability of a few monitored populations invite complacency, our continued inability to document more than trivial seedling recruitment (Menges et al., 2008) is a cause for concern. In the two populations with the longest monitoring records (Pace-Aldana et al., 2006; Menges et al., 2008), researchers have found that although mortality is low, it exceeds recruitment. More frequent burning of sites supporting scrub plum populations, particularly sandhills, may result in a larger number of germinable seeds entering the seed bank. Even low levels of seedling recruitment could promote the long-term persistence of populations of this endangered species.

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