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EFFECTS OF SELF-POLLINATION AND OUTCROSSING WITH CULTIVATED PLANTS IN SMALL NATURAL POPULATIONS OF AMERICAN GINSENG, *PANAX QUINQUEFOLIUS* (ARALIACEAE)[1]

EMILY H. MOONEY[2] AND JAMES B. MCGRAW

West Virginia University, Department of Biology, P.O. Box 6057, Morgantown, West Virginia 26506 USA

For rare plants, self-pollination and inbreeding can increase in small populations, while unusual levels of outcrossing can occur through restoration efforts. To study both inbreeding and outcrossing, we performed experimental pollinations using *Panax quinquefolius* (American ginseng), a wild-harvested plant with a mixed mating system. For inbreeding, plants were either cross-pollinated within the population or self-pollinated, which resulted in a higher proportion of seeds from self-pollinated flowers. For outcrossing, wild plants were either cross-pollinated within the population or with cultivated plants from West Virginia or Wisconsin. Offspring of all crosses were followed for 4 yr. Two-yr-old seedlings from self-pollination had 45% smaller leaf areas and 33% smaller heights relative to those from cross-pollination. Leaf area is a positive predictor of longer-term survival in wild populations. Our results suggest inbreeding depression, which is unexpected in this self-fertile species. Seedlings from crosses with cultivated plants had 127% greater leaf area and 165% greater root biomass relative to outcrosses within the population. The accelerated growth suggests genetic differences between wild and cultivated populations, but outbreeding depression may not appear until later generations. Assessment of the ultimate fitness consequences of introducing cultivated genotypes requires monitoring over longer time periods.

Key words: Araliaceae; cultivated genotypes; inbreeding depression; *Panax quinquefolius*; West Virginia.

The determinants of plant reproductive success can vary as population sizes and densities fluctuate with environmental stochasticity. For example, self-pollination would prevail when pollinators are scarce or conspecifics too few. At other times, increased population sizes and densities would provide more opportunities for outcrossing. Human activities may also affect population size and densities in some plant species. For populations reduced in size by humans, inbreeding depression is a conservation concern due to the increased likelihood of self-pollination and mating between relatives (Ellstrand and Elam, 1993; Crnokrak and Roff, 1999; Hedrick and Kalinowski, 2000; Keller and Waller, 2002). Species without an evolutionary history of inbreeding are likely to harbor deleterious recessives (Byers and Waller, 1999), which can further accelerate population extinction (Gilpin and Soulé, 1986). At the same time, humans may work to increase population sizes through restoration efforts. Besides supplementing the number of individuals, restoration may be motivated by examples of "genetic rescue," in which the introduction of new genotypes has relieved inbreeding depression (Richards, 2000; Newman and Tallmon, 2001; Tallmon et al., 2004). The introduction of new individuals to populations of rare plants poses its own risks, for example, the introduction of disease and outbreeding depression (Storfer, 1999; Hufford and Mazer, 2003). Outbreeding depression can occur if "rescuers" introduce genes selected elsewhere or if outcrossing leads to the breakup of locally adapted gene complexes (Lynch, 1991; Templeton, 1991; Schierup and Christiansen, 1996). Because of the potential consequences of small population size and well-intentioned restoration attempts, a rare plant can be simultaneously at risk for inbreeding and outbreeding depression.

To examine these issues in natural populations, we selected *Panax quinquefolius* L. (Araliaceae), American ginseng, a plant for which novel levels of both inbreeding and outcrossing are possible because of human activities. Harvest of the medicinal root and browse by overabundant white-tailed deer (*Odocoileus virginianus*) both reduce local population size in *P. quinquefolius* (Van der Voort et al., 2003; McGraw and Furedi, 2005; Van der Voort and McGraw, 2006). In general, habitat destruction (Drayton and Primack, 1996), logging (Duffy and Meier, 1992), and invasive species (Miller and Gorchov, 2004) all negatively affect understory species and are likely to reduce population sizes for *P. quinquefolius* as well. Given that roughly 15 million plants were removed from the wild in 2004 (USFWS, 2005), harvest is a dominant feature in the population biology of *P. quinquefolius*. The scale of harvest led to the listing of *P. quinquefolius* in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1973 (Robbins, 2000). Harvest can reduce genetic diversity in *P. quinquefolius* (Cruse-Sanders et al., 2005), and low levels of allozyme diversity in the wild have raised concerns about inbreeding depression in remnant populations (Cruse-Sanders and Hamrick, 2004a; Grubbs and Case, 2004).

The potential for inbreeding depression depends on the evolutionary history of inbreeding, which for plants is primarily determined by breeding system (Loveless and Hamrick, 1984; Husband and Schemske, 1996). *Panax quinquefolius* has a mixed mating system: flowers are self-compatible, but previous studies have found various levels of protandry (Carpenter and Cottam, 1982; Lewis and Zenger, 1983; Schlessman, 1985), which is one mechanism to reduce self-pollination (Bawa and Beach, 1981). Mixed mating systems seemingly defy theories based on inbreeding depres-

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[2] Author for correspondence (e-mail: emooney@muhlenberg.edu)

sion, which predict species to be either entirely self-fertilizing or outcrossing (Lande and Schemske, 1985). The prevalence of mixed mating systems suggests that pressures other than inbreeding depression alone govern reproductive success (Goodwillie et al., 2005). One of the main factors recognized to promote mixed mating systems is the reproductive assurance that self-compatibility provides when pollinators or nonself pollen are scarce (Jain, 1976). In *P. quinquefolius*, there is evidence from small experimental populations that seed set can be reduced by fewer pollinator visits (Hackney and McGraw, 2001). In contemporary populations, inbreeding coefficients estimated by molecular markers are consistent with high levels of inbreeding (Cruse-Sanders and Hamrick, 2004b; Grubbs and Case, 2004). However, evidence from herbarium specimens suggests that population sizes have declined substantially over the last 150 yr (Case et al., 2007). If the high levels of inbreeding associated with small population sizes are recent phenomena, then inbreeding depression is theoretically more likely (Lande and Schemske, 1985; Byers and Waller, 1999).

Some natural populations of *P. quinquefolius* may be subjected to unusual levels of outcrossing through the introduction of seeds from cultivation. *Panax quinquefolius* has been cultivated for approximately 120 yr, even though roots produced in cultivation are readily discernable from higher-priced wild roots (Carlson, 1986). Agricultural practices for *P. quinquefolius* range in intensity from 100 ha fields where plants are grown under artificial shade (Proctor and Bailey, 1987) to "wild-simulated" production that can simply be the broadcast of seeds into a forested area (Beyfuss, 1998). At intermediate intensity, "woods grown" can refer to the production of roots in prepared beds under a natural overstory (Beyfuss, 1998). *Panax quinquefolius* plants in cultivation produce copious amounts of seeds relative to wild plants, and these seeds are frequently sold on the open market (Schluter and Punja, 2000). Cultivated seeds could be introduced to the forests alongside wild populations in several ways: by growers via woods-grown and wild-simulated seeding or by managers and harvesters planting seeds when attempting to "restock" forests (USFWS, 2005). Although cultivated *P. quinquefolius* is not considered to be domesticated, there are potentially important differences between cultivated and wild populations. Genetic markers have shown that cultivated populations are dramatically more diverse than those in the wild; this likely reflects the origin of cultivated populations from seeds exchanged among growers (Bai et al., 1997; Grubbs and Case, 2004). In addition, random amplified polymorphic DNA (RAPD) markers have been found that are unique to cultivated plants from Wisconsin (Lim, 2004), where the majority of *P. quinquefolius* is grown in the United States (Hsu, 2002). Efforts to select for desirable traits are preliminary in *P. quinquefolius* (Canter et al., 2005), but unintentional selection often results from the interaction with humans in a cultivated environment (Zohary, 2004). Genotypes selected across many generations for success in a high-nutrient, low-stress cultivated environment are likely to be different from those of wild relatives.

If flowering times overlap, wild and cultivated plants could hybridize. Possible outcomes of such hybridization are suggested by studies from other plant species. In the first generation (F1), outbreeding depression may arise from the introduction of cultivation-selected genes that dilute the effects of locally adapted genes (Montalvo and Ellstrand, 2001; Hufford and Mazer, 2003). Subsequent generations (F2 or later) may show additional evidence of outbreeding depression as recombination breaks down coadapted gene complexes (Lynch, 1991; Fenster and Galloway, 2000; Hufford and Mazer, 2003). Rather than fitness being reduced in hybrids, cultivated–wild hybrids could actually outperform local genotypes (Hufford and Mazer, 2003). Given significant fitness advantages, cultivated–wild hybrids could eventually replace local genotypes, i.e., genetic swamping (Hufford and Mazer, 2003).

Our objective was to assess the first generation consequences of inbreeding and outcrossing for offspring fitness in *P. quinquefolius*. To examine the results of inbreeding, we produced offspring by self-pollinating plants and crossing plants with individuals at two distance classes within wild populations. To assess the consequences of outcrossing with cultivated genotypes, we produced offspring by pollinating flowers of wild maternal plants with pollen from cultivated plants. Progeny resulting from both sets of crosses were planted in natural conditions, and their germination, growth and survival were followed for 4 yr. Though additional fitness consequences would likely appear in later generations, the average of 5–10 yr required for *P. quinquefolius* to reach reproductive maturity made performing subsequent crosses unfeasible. Because *P. quinquefolius* is a long-lived species, we wanted to determine how early life-cycle characteristics might influence survival over longer time frames, i.e., beyond the 4 years that we observed offspring of experimental crosses. For this purpose, we took advantage of fitness data collected as part of an ongoing monitoring study of eight wild populations in West Virginia, USA.

MATERIALS AND METHODS

Reproductive biology—Populations of *P. quinquefolius* are distinctly stage-structured, with individuals being readily classified on the basis of their leaf number. Reproduction in *P. quinquefolius* takes place through seed, with vegetative reproduction rare in wild populations. Seedling and one-leaved plants generally grow to become juvenile (two-leaved) plants. Juvenile plants may or may not be reproductive, whereas three- and four-leaved plants are almost all reproductive. A single immature inflorescence is present on plants upon emergence of aboveground portions in early May. These inflorescences develop into a simple umbel containing several small, hermaphroditic flowers (Schlessman, 1987). Anthesis takes place in mid June and continues as flowers mature centripetally within an inflorescence (Lewis and Zenger, 1983; Schlessman, 1985, 1987). Flowers of *P. quinquefolius* are self-compatible, allowing for pollination to occur both within flowers (autogamy) and within an inflorescence (geitonogamy) (Lewis and Zenger, 1983; Schlessman, 1985). Several generalist pollinators also visit inflorescences of *P. quinquefolius*, most importantly syrphid flies and halictid bees (Duke, 1980; Lewis and Zenger, 1983; Schlessman, 1985). Flowers contain a single ovary with one to two (rarely three) carpels each (Schlessman, 1985, 1987). A single *P. quinquefolius* flower may produce as many as one seed per carpel, but three-seeded fruits are rarely observed in wild populations (Carpenter and Cottam, 1982; Schlessman, 1985; Schluter and Punja, 2000). Fertilized flowers mature into red fruits by late August to early September, with population to population variation in ripening phenology (McGraw et al., 2005).

Study populations—In early June 2003, we mapped and marked four populations located in the understory of mixed mesophytic forests outside of Morgantown, West Virginia (Fig. 1). All populations were initially located by surveys conducted as parts of an earlier study of the distribution of *P. quinquefolius* (McGraw et al., 2003). Each population is given an acronym in this publication to protect the details of its specific location (CB, CL, FC1, and FC2). The number of reproductive individuals varied among the four populations (Table 1); plants were patchily distributed across approximately 1-ha areas, as is typical of wild populations of *P. quinquefolius* (Cruse-Sanders

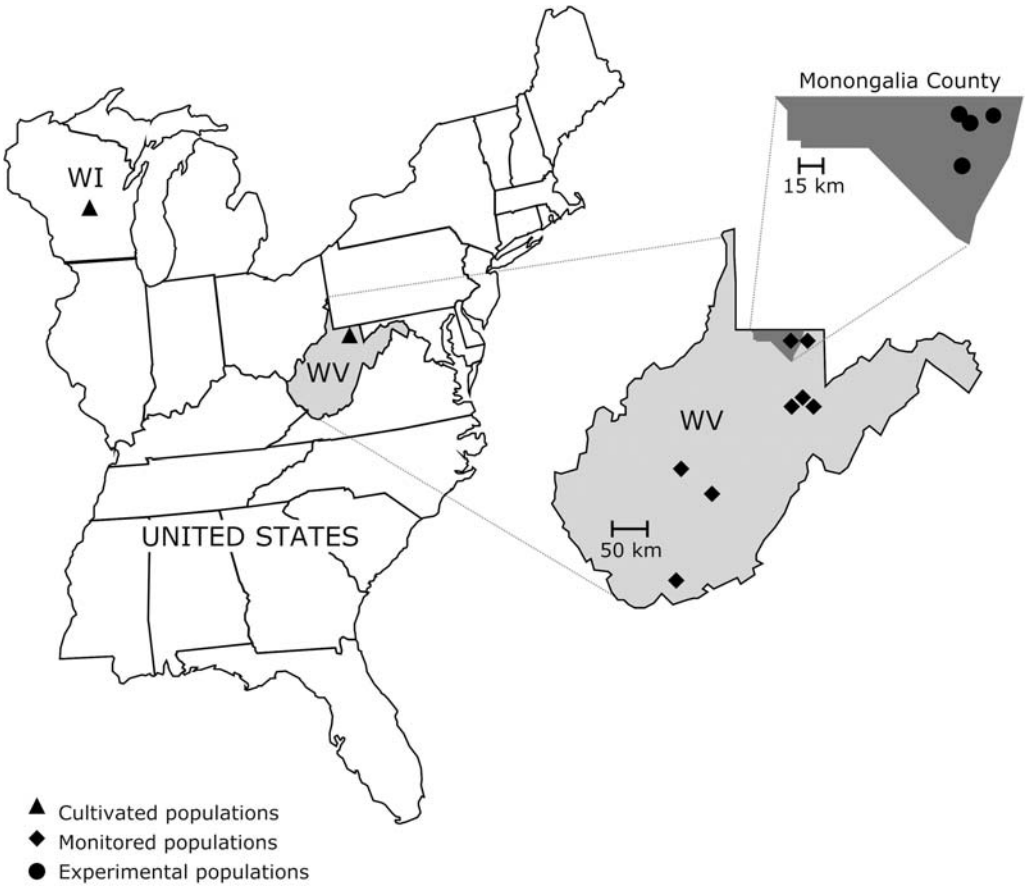

Fig. 1. Map of the eastern United States with origins of the cultivated plants used as pollen sources from farms in Marathon County, Wisconsin (WI) and Preston County, West Virginia (WV). The inset maps show the locations of eight wild populations where seedlings were monitored from 2002 to 2006 in WV and the locations of populations where experimental pollinations were performed near Morgantown, WV.

and Hamrick, 2004b). Reproductive plants in each population were placed into individual enclosures made of poultry netting to exclude aboveground herbivores (e.g., white-tailed deer). The largest population (FC1) contained 49 reproductive plants, such that we were able to apply both inbreeding and outcrossing pollination treatments.

Pollination treatments—We sought to compare offspring of self-pollinations to those created by crosses with plants of differing relatedness to the maternal plant. To determine relatedness in situ, we used allozyme information from previous analyses of the fine-scale genetic structure in populations of *P. quinquefolius* (Cruse-Sanders and Hamrick, 2004b). Spatial distribution is patchy, and plants within 2 m of each other have significant levels of relatedness in most populations (Cruse-Sanders and Hamrick, 2004b). We measured distances between plants using an electronic measuring tool (Sonin Multi-Measure Combo PRO, Sonin, Inc, Charlotte, North Carolina, USA). Few reproductive plants overall were located within 2 m of each other in our study populations. To be sure we crossed close relatives, plants within a mean of 3.45 m from each other were classified as being within the same patch. Plants at greater distances were classified as being in different patches; the mean distance between plants in different patches was 24.24 m. Individuals in populations CB, CL, and FC1 were randomly assigned to act as maternal plants and receive one of the three pollination treatments: self-pollination, crosses with a plant within their patch, and crosses with a plant in a different patch. Unfortunately, small population sizes made it unfeasible to perform interpopulation crosses as well. Because of the proximity of flowers within an inflorescence, each inflorescence (one per maternal plant) was assigned a single pollination treatment.

In the outcrossing pollination treatments, we sought to compare the

TABLE 1. Number of *Panax quinquefolius* plants in each of the six pollination treatments among four study populations. Individual plants had a single inflorescence, which was assigned one pollination treatment. The number of individual flowers are indicated in parentheses. Cultivated plants used in the outcrossing study as pollen donors came from either West Virginia (WV) or Wisconsin (WI).

		Inbreeding			Outcrossing			
Population	Control	Self-pollinated	Within patch	Between patch	Within population	WV cultivated	WI cultivated	Total
CB	1(4)	6(26)	7(35)	9(43)	—	—	—	23(108)
CL	2(9)	4(37)	6(28)	9(50)	—	—	—	21(124)
FC1	5(27)	2(12)	5(20)	7 (38)	12(55)	11(57)	7(49)	49(258)
FC2	4(26)	—	—	—	3(30)	8(64)	5(46)	20(166)
Total	12(66)	12(75)	18(83)	25(131)	15(85)	19(121)	12(95)	113(656)

TABLE 2. Number of one-seeded fruits, number of two-seeded fruits, and total number of seeds produced by experimental pollination of *Panax quinquefolius* plants in four populations. Cultivated plants used in the outcrossing study came from either West Virginia (WV) or Wisconsin (WI).

		Inbreeding			Outcrossing			
Population	Seeds in fruit	Self-pollinated	Within patch	Between patch	Within population	WV cultivated	WI cultivated	Total seeds
CB	One	6	2	3	—	—	—	11
	Two	0	0	0	—	—	—	
CL	One	4	0	3	—	—	—	11
	Two	2	0	0	—	—	—	
FC1	One	—	—	—	12	14	2	52
	Two	—	—	—	5	7	0	
FC2	One	2	0	1	6	4	14	55
	Two	0	0	0	3	2	9	
Total seeds		16	2	7	34	36	34	129

offspring of crosses within the population to those with cultivated plants. Outcrosses within the population were achieved using pollen from other plants in the population, though distances between plants were not measured. To provide pollen at the two levels of outcrossing with cultivated plants, we obtained reproductively mature plants as bare roots from cultivated sources in West Virginia and Wisconsin. Seeds from growers in both Wisconsin and West Virginia are commercially available, so they represent populations that could be used for restoration purposes. Wisconsin was chosen as one source because the majority (>75%) of commercially cultivated *P. quinquefolius* in the U.S. originates from farms in Wisconsin (Hsu, 2002). The cultivated roots were planted into standard potting soil in 10-inch Deepots (Stuewe and Sons, Corvallis, Oregon, USA). When not being used to pollinate the study populations, cultivated plants were kept separately in a naturally shaded location to avoid inadvertent pollen transfer.

Pollination process—Before the onset of flowering, inflorescences were bagged with small caps made of fine nylon mesh; this bagging excluded natural pollinators, which might visit flowers and deposit pollen. Flowering commenced between 18 June and 1 July, and the final flowers within inflorescences opened between 23 July and 26 July in the four populations. As each flower opened, stamens were removed using fine forceps. Pollen was applied by physically dusting the stigmas with an anther from a plant matching the corresponding pollination treatment. For example, stigmas of plants assigned the WV cultivated pollination treatment were dusted with pollen from a stamen of a WV-cultivated plant. Plants that were self-pollinated were similarly treated; that is, stamens were removed from their flowers, but were then used to dust their own stigmas. Populations were visited every 24 to 48 h during the flowering period to emasculate flowers and apply the pollination treatments. Control plants in each population had their stamens removed without subsequent pollination to test whether pollination occurred before emasculation or whether pollen reached the stigma despite bagging.

Seed production and planting—Previous researchers have counted the number of carpels per flower by counting stigmas under magnification, with one stigma per carpel (Schlessman, 1985; Schluter and Punja, 2000). However, differentiating between single stigmas vs. stigma lobes is difficult in situ, so we cannot determine seed set on the basis of seeds per carpel. The majority of fruits were one-seeded, and no three-seeded fruits were observed on any plant (Table 2). Seed production was low, with a mean of 18.9% of pollinated flowers producing any seeds across all pollination treatments. To examine whether there were differences in seed production, we compared the proportion of flowers producing any seeds among pollination treatments using log-likelihood tests (Sokal and Rohlf, 1995).

Because fruit maturity can significantly affect germination and ripening phenology varies between populations (McGraw et al., 2005), individual fruits were collected only upon completion of ripening from 9 August to 8 September. We constructed seed cages from 10-cm sections of 7.5 cm diameter acrylonitrile-butadiene-styrene piping with heavy-duty nylon screening attached to the bottom. The cages were filled with soil that came from the site and had been carefully examined to remove any native seeds. Each seed was planted 3 cm deep into its own seed cage, which was labeled with an engraved aluminum nail. The seed cages were then placed within 1 m of the maternal plant to simulate natural dispersal distances. Seed cages were placed within enclosures made of poultry netting to prevent herbivory of seedlings by white-tailed deer.

Germination—*Panax quinquefolius* seeds possess deep, simple morphophysiological dormancy and remain in the soil for at least 20 mo before germinating (Anderson et al., 1993; Baskin and Baskin, 1998). Given this dormancy period, the majority of seeds were expected to germinate in 2005, i.e., the second spring following planting. Nevertheless, we monitored the seed cages monthly from May to September 2004 to replace the poultry netting enclosures or replant seed cages if disturbed. At the start of May 2005 and 2006, we checked the seed cages for emergence of seedlings from the soil. In August 2006, the soil from the seed cages where no seedling appeared in 2005 or 2006 was carefully sifted to recover any remaining seeds. If a seed was present, we tested for viability using a 0.1% tetrazolium solution (Baskin and Baskin, 1998).

Growth and survival—We measured stem height (from soil to base of leaf) once emergence of new seedlings was complete in May 2005. We photographed the leaves of each seedling against a white background. By including a ruler for scale, we were able to convert the pixels of each digital leaf image into an area measurement using NIH Image v.1.63 software (U.S. National Institutes of Health, 2005). The seedlings were monitored bimonthly until September to follow their fates over the 2005 growing season.

In May 2006, we measured the leaf area and stem height for any new seedlings and reemerging 1-yr-old plants using the same techniques as described previously. In July 2006, we also estimated chlorophyll content of leaves using a Minolta SPAD 502 Chlorophyll meter (Konica Minolta Group, Hong Kong). We used the leaf area measurements from plants present in both 2005 and 2006 to determine the relative growth rate on a leaf area basis:

$$\mathrm{DRGR_{LA}} = \frac{\ln A_2 - \ln A_1}{t_2 - t_1},$$

for which A_2 is the leaf area in 2006, A_1 is the leaf area in 2005, and the time interval would be 1 year (McGraw and Garbutt, 1990).

In August 2006, living seedlings were removed from the surrounding soil and separated into root and shoot portions. Sections of lateral root tissue (1 cm long) were tested for mycorrhizal colonization using trypan blue staining (Phillips and Hayman, 1970). The sections of stained root were examined under a dissection microscope at 60× for the presence of mycorrhizal structures. We also determined root and shoot biomass after the tissue was dried in an oven at 80°C for 48 h. Seed cages where seedlings had germinated and apparently "died" were carefully searched for any remaining root material; if a root had an apical bud present on the rhizome, it was considered viable.

Seedlings in wild populations—To determine the significance of leaf area and stem height measures for longer-term survival, we used data from eight populations in West Virginia monitored since 2002. Individual plants are measured twice yearly following the techniques outlined by McGraw and Furedi (2005). New seedlings were identified in these populations by the presence of a persistent seed coat. By following the fate of seedlings in 2002, we used logistic regressions to determine the relationship of initial seedling stem height and leaf area to survival over 4 yr (Sokal and Rohlf, 1995). The analyses were repeated for 1-yr-old plants in 2003 (i.e., new seedlings from

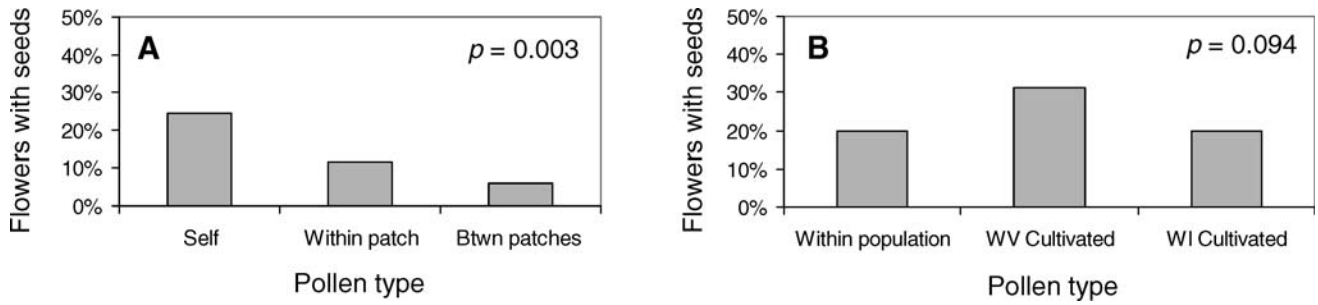

Fig. 2. Seed set measured as the percentage of *Panax quinquefolius* flowers producing seed in August 2005 for (A) inbreeding pollination treatments and (B) outcrossing pollination treatments.

previous year) to determine the importance of measures taken at this time for subsequent survival. Using leaf area measures from 2002 and 2003, we also used logistic regression to determine how $DRGR_{LA}$ predicted survival to 2006 (Sokal and Rohlf, 1995).

Data analysis—Within the inbreeding and outcrossing studies, data were pooled across populations because of low sample sizes in certain population–pollination treatment combinations. Because of the relatively few seeds (33 total) produced by the inbreeding study, the within- and between-patch pollination treatments were combined. Subsequent analyses compared seeds produced by self-pollination to those produced by cross-pollination, regardless of distance of pollen donor from maternal plant. This also allowed the incorporation of seeds produced by the within-population crosses for the outcrossing study at the FC1 population. Because no comparable self-pollinated seeds were produced at FC2, seeds produced by the within-population crosses for this population were not included.

Germination frequency and year of germination were compared among the seeds produced by the pollination treatments using log-likelihood tests for both the inbreeding and outcrossing studies. For those seedlings that germinated in 2005 or 2006, size measures (leaflet length and width, leaf area, and stem height) were compared among pollination treatments using a one-way ANOVA (Sokal and Rohlf, 1995). Survival of seedlings to 1-yr-old plants in 2006 was compared among pollination treatments using a log-likelihood test. Size measures of 1-yr-old plants measured in 2006 and their $DRGR_{LA}$ were both compared among pollination treatments using one-way ANOVAs. Finally, we compared root and shoot biomass of seedlings among pollination treatments for both inbreeding and outcrossing studies using one-way ANOVAs (Sokal and Rohlf, 1995). For the outcrossing study, results of one-way ANOVAs with significant differences were examined further using Tukey–Kramer honestly significant difference (HSD) to detect specific differences among groups. All statistical tests were completed using SAS JMP version 6.0 (SAS Institute, 2005), and significance was recognized when $P < 0.05$.

RESULTS

Seed production—For the control plants, 3.1% and 4.1% of flowers produced seeds in the populations used for the inbreeding and outcrossing studies, respectively. Self-pollination produced a greater proportion of flowers that set any seeds compared to crosses at either distance within the population (df $= 2$, $\chi^2 = 11.97$, $p = 0.0025$; Fig. 2A). There was a trend ($0.05 < p < 0.10$) for the proportion of flowers producing seeds to vary among the outcrossing pollination treatments (df $= 2$, $\chi^2 = 4.73$, $p = 0.094$; Fig. 2B). Flowers pollinated using WV-cultivated pollen tended to have a slightly higher rate of seed production than those pollinated by either plants within the population or by WI-cultivated plants.

Germination—Three of 11 seeds produced by one plant crossed with WV-cultivated pollen germinated in May 2004; however, most seeds germinated in May 2005 as expected. In 2005, seeds produced from self-pollination germinated at a rate similar to those produced by crosses within the population (df $= 1$, $\chi^2 = 1.00$, $p = 0.32$). Likewise, there was no difference in germination rate of seeds produced by the outcrossing treatments (df $= 2$, $\chi^2 = 3.97$, $p = 0.14$). In May 2006, two new seedlings appeared, one produced by cross-pollination in the inbreeding pollination treatments and one produced by crosses with WV-cultivated plants among the outcrossing pollination treatments. When the seed cages were exhumed in August 2006, five intact (e.g., endocarp not broken) seeds were recovered from among the 27 cages that had no germination in either 2005 or 2006. All intact seeds were the offspring of crosses with WV-cultivated plants and were viable.

Size, growth, and survival of experimental seedlings—No differences in stem height, leaflet length and width, or leaf area were observed between seedlings produced by different pollination treatments in either the inbreeding or outcrossing studies in the first year following germination. Likewise, there was no difference in survival over the first season of growth (2005) between seedlings produced by self-pollination and by outcrossing (df $= 1$, $\chi^2 = 0.29$, $p = 0.59$). Nor were there differences in survival over the first season among seedlings produced by the outcrossing pollination treatments (df $= 2$, $\chi^2 = 1.76$, $p = 0.42$).

Altogether, most seedlings (83.7%) that survived the first season of growth reemerged as 1-yr-old plants in May 2006; though a majority of these plants were one-leaved, a few individuals emerged as two-leaved juveniles. A higher number of offspring produced by crosses with WI-cultivated plants emerged as juveniles than offspring produced by either crosses within the population or with WV-cultivated plants (df $= 2$, $\chi^2 = 10.67$, $p = 0.0048$).

For both the inbreeding and outcrossing studies, offspring produced by crosses farther from the wild maternal plant produced larger offspring. In the inbreeding study, 1-yr-old plants produced by cross-pollination had 44.9% greater leaf area than those produced by self-pollination (ln transformed, $F = 5.49$, $P = 0.031$; Fig. 3A). Seedlings produced by crosses with WI-cultivated plants had 127.3% greater leaf area than seedlings produced by crosses within the population (square root transformed, $F = 4.38$, $P = 0.019$; Fig. 3B). In the inbreeding study, outcrossed offspring also had 33.0% greater stem height than offspring produced by self-pollination ($F = 4.43$, $P = 0.0495$; Fig. 3C). There were no significant differences in stem height among offspring produced with any of the outcrossing pollination treatments ($F = 0.44$, $P =$

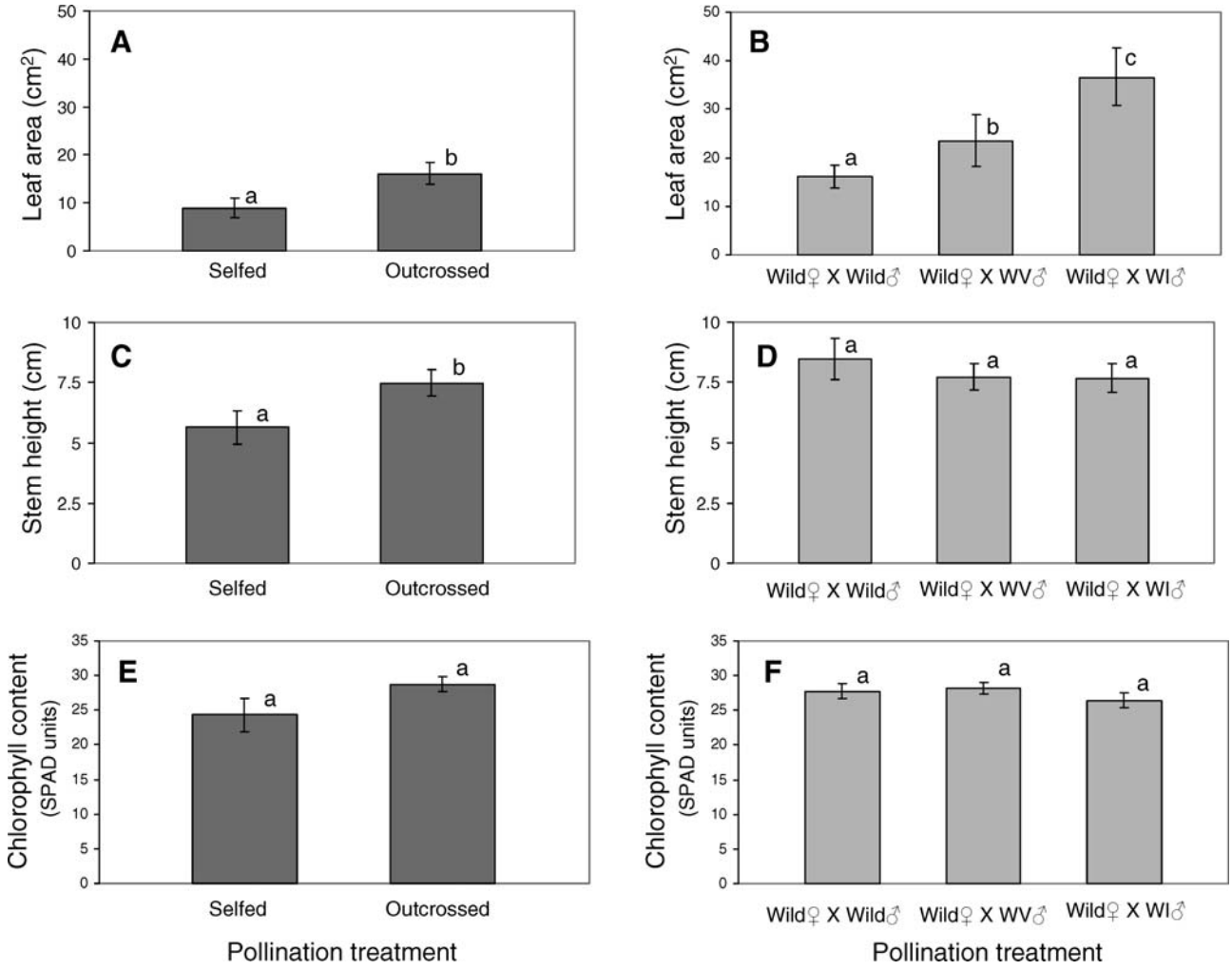

Fig. 3. Effects of pollination treatments on leaf area (A, B), stem heights (C, D) and chlorophyll content (E, F) of 1-yr-old *Panax quinquefolius* seedlings. Left panels (A, C, E) show data from the inbreeding pollination treatments, and right panels (B, D, F) show data from the outcrossing pollination treatments. Means and standard errors were back-transformed when applicable. Letters above bars show differences determined by Tukey–Kramer HSD procedure.

0.65; Fig. 3D). There was a trend toward higher chlorophyll content in outcrossed offspring relative to those produced by self-pollination ($F = 3.68$, $P = 0.073$; Fig. 3E). No differences in chlorophyll content were observed among offspring produced by the outcrossing treatments ($F = 0.90$, $P = 0.42$; Fig. 3F).

Survival to August 2006 was similar between the inbreeding or among the outcrossing pollination treatments. There was also no difference in relative growth rate on a leaf area basis ($DRGR_{LA}$) between offspring produced by self-pollination or cross-pollination ($F = 1.47$, $P = 0.24$). However, the $DRGR_{LA}$ was significantly higher in the offspring of crosses with WI than either the offspring of crosses within the population or with WV-cultivated plants ($F = 9.01$, $P = 0.0007$). Remarkably, one individual produced by crosses with WI-cultivated plants flowered and produced a single viable seed.

Most differences in $DRGR_{LA}$ were also reflected in the biomass measurements. In the inbreeding study, biomass of roots did not differ between offspring produced by outcrossing and self-pollination (Fig. 4A). Offspring produced by crosses with WI-cultivated plants had 164.8% greater root biomass than outcrossing within the population ($F = 3.75$, $P = 0.034$; Fig. 4B). Shoot biomass did not differ between offspring of self-pollination and cross-pollination (Fig. 4C). However, offspring produced by crosses with WV-cultivated plants tended toward greater shoot biomass than offspring of crosses within the population or with WI-cultivated plants ($F = 2.94$, $P = 0.074$; Fig. 4D). Because many roots lacked lateral roots, fewer roots were available for mycorrhizal testing. All the roots tested ($N = 12$) from offspring produced by either self-pollination or cross-pollination had evidence of mycorrhizal colonization (data not shown). Despite small sample sizes, seedlings produced by crosses within the population had a significantly higher rate of mycorrhizal colonization (87.5%) than either set of offspring produced by crosses with cultivated plants, WI = 16.67% and WV = 42.86% ($df = 2$, $\chi^2 = 7.45$, $p = 0.024$; Fig. 5).

Size, growth, and survival of wild seedlings—Among the eight wild populations monitored, we were able to assess the

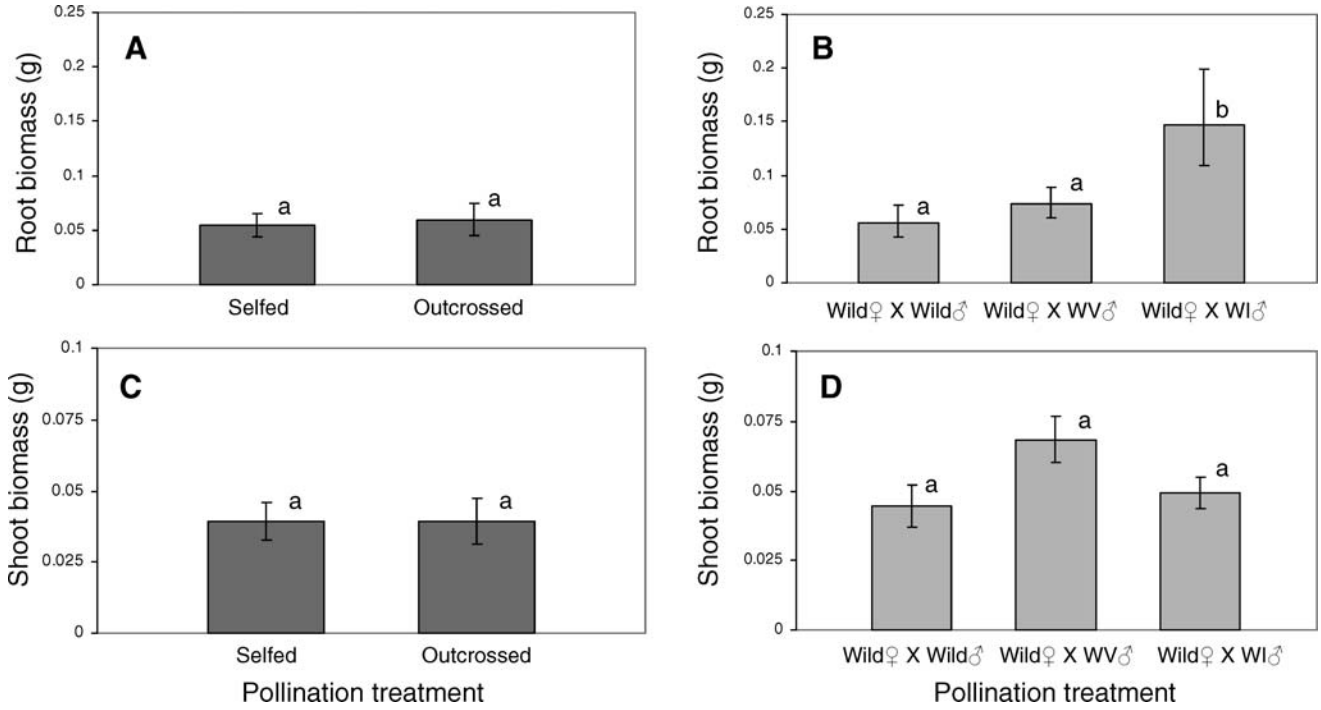

Fig. 4. Effects of pollination treatments on root biomass (A, B) and shoot biomass (C, D) of 1-yr-old *Panax quinquefolius* seedlings. Right panels show data from the inbreeding pollination treatments, and left panels show data from the outcrossing pollination treatments. Mean and standard errors were back-transformed when applicable. Letters above bars show differences determined by Tukey–Kramer HSD procedure.

survival of 78 new seedlings that germinated in 2002. Leaf area of new seedlings was a significant positive predictor of survival to 2006 ($df = 1$, $\chi^2 = 5.05$, $p = 0.025$; Fig. 6A). Also, there was a trend suggesting a positive relationship between survival to 2006 and stem height of new seedlings in 2002 ($df = 1$, $\chi^2 = 3.27$, $p = 0.071$). Because of 39.7% mortality in the first year following germination, fewer 1-yr-old plants were available for analysis; however, leaf area was again a significant positive predictor of survival to 2006 ($df = 1$, $\chi^2 = 4.65$, $p = 0.031$; Fig. 6B). No relationship between stem height of 1-yr-old plants and survival to 2006 was found. $DRGR_{LA}$ measured from 2002 to 2003 was not a significant predictor of survival to 2006 ($df = 1$, $\chi^2 = 0.91$, $p = 0.34$).

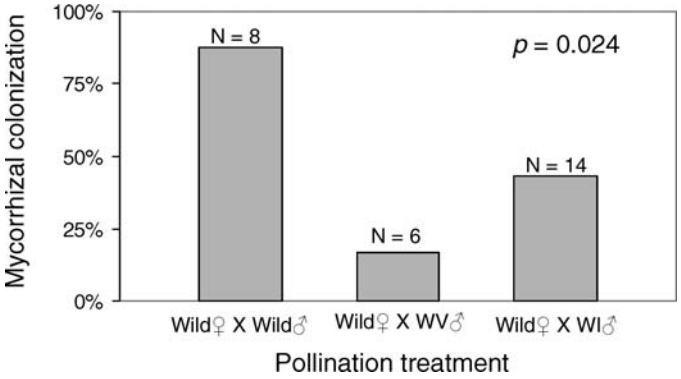

Fig. 5. The frequency of mycorrhizal colonization in roots of 1-yr-old *Panax quinquefolius* seedlings produced by outcrossing pollination treatments.

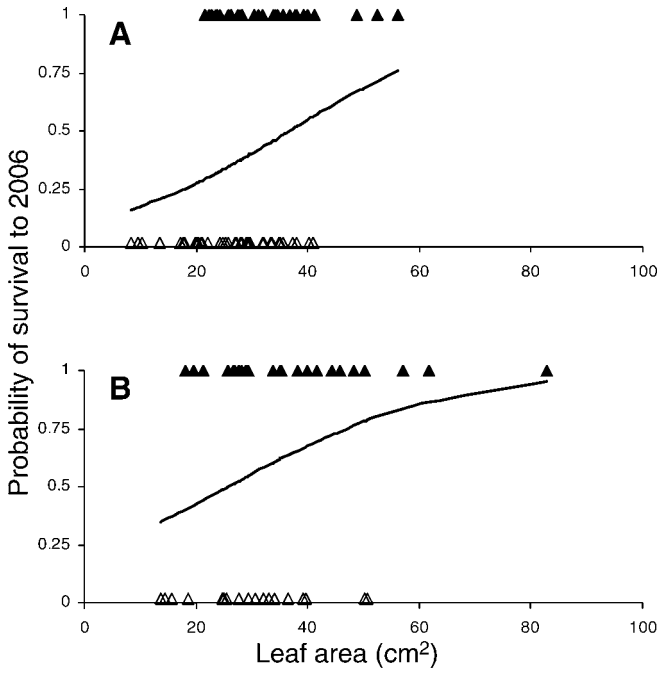

Fig. 6. Curves showing results from logistic regression of leaf area on survival to 2006 for (A) new seedlings measured in 2002 and (B) 1-yr-old seedlings measured in 2003. Data points are from seedlings from eight wild populations of *Panax quinquefolius*, with seedlings that survived shown as filled triangles and seedlings that died shown as clear triangles.

DISCUSSION

Inbreeding pollination treatments—One potentially important fitness difference we observed was the increased probability of seed production by self-pollinated flowers relative to cross-pollinated flowers. This difference contrasts with previous studies of *P. quinquefolius* that have reported equal seed set between experimentally self-pollinated and cross-pollinated flowers (Lewis and Zenger, 1983; Schlessman, 1985). Our seed production results need to be carefully interpreted for several reasons. Namely, we were only able to assess seed production on a per-flower basis, which does not account for the potential of some flowers to produce variable numbers of seeds. Though the majority of fruits we observed were one-seeded, fruits can develop from flowers with a single carpel or through selective postpollination events in flowers with more than one carpel (Schlessman, 1985). Generally, processes affecting seed set may be divided sequentially into variation in pollination (pollen reaching stigmas), fertilization (pollen tube growth through stylar tissue), and seed maturation (development of fertilized ovules) (Lyons et al., 1989). In one previous study of cultivated *P. quinquefolius*, researchers did find that the amount of pollen reaching stigmas influenced seed set (Hackney and McGraw, 2001). All flowers in this study were consistently hand-pollinated upon anthesis; thus, pollen transfer was assumed to be equal among treatments. Differences in pollen tube formation and growth would reflect maternal and paternal interaction, whereas seed maturation could be attributed to maternal resource partitioning or could be considered an early measure of offspring fitness (Lyons et al., 1989; Waser and Price, 1993). Because pollen tube growth and selective embryo abortion were not determined in situ, we are limited in how we can interpret differences in seed set with respect to inbreeding depression. Nevertheless, our results demonstrate the success of self-pollination for seed production in *P. quinquefolius*.

Seed production reflects the interaction of maternal, paternal, and offspring fitness, but germination can more directly indicate offspring fitness. Most seeds followed the expected pattern by germinating after two winters of dormancy (Baskin and Baskin, 1998; McGraw et al., 2005). The lack of difference between seeds produced by self-pollination vs. cross-pollination suggests no inbreeding depression in this trait. Previous generations of close inbreeding may have effectively purged any deleterious alleles acting at this stage (Husband and Schemske, 1996; Schierup and Christiensen, 1996). Alternatively, the relatively benign planting environment could have mitigated any germination differences. Although seeds were planted in natural soil, they were planted at an optimal depth for germination of *P. quinquefolius* seeds (McGraw et al., 2005). Studies in other species have shown that favorable environments can reduce the effects of inbreeding (Dudash, 1990; Heschel and Paige, 1995).

In the year of germination, survival and size of offspring were similar, but differences in the leaf area and stem height appeared in the second year of growth. Differences among offspring produced by controlled crosses have appeared only later in life in studies of other perennial plants (Wolfe, 1993; Waser and Price, 1994; Kephart et al., 1999; Luitjen et al., 2002). Generally, differences are likely to increase with time as the expression of the offspring genome overrides maternal genetic effects and environmental variation (Wolfe, 1993; Husband and Schemske, 1996). Size differences rather than survival differences at this stage follows theoretical predictions: in self-fertile species, inbreeding depression is likely due to many loci of small effect, rather than easily purged, highly deleterious recessives (Charlesworth and Charlesworth, 1987; Schierup and Christiansen, 1996). In a majority of studies of self-fertile species reviewed by Husband and Schemske (1996), inbreeding depression was expressed in growth and reproduction rather than in survival. Another explanation for the lack of observed differences in survival could be that deleterious alleles are fixed in the study populations. In this case, inbreeding depression would only be apparent in interpopulation crosses because offspring of crosses within populations would be similarly inbred (Keller and Waller, 2002). Nevertheless, we see some evidence of inbreeding depression, suggesting that complete fixation of deleterious alleles has not occurred in these wild populations. The differences in stem height and leaf area between seedlings of self-pollination and cross-pollination were not reflected in their biomass; this result suggests that early size differences did not affect carbon acquisition. However, early size does seem to influence longer-term survival for *P. quinquefolius* seedlings, as we observed from seedlings in the eight wild populations we monitored. If the smaller offspring of self-pollination have reduced survival rates later in life relative to those of cross-pollination, then this would suggest inbreeding depression in a trait important for population growth.

The results from the inbreeding pollination treatments indicate that, while self-pollination may provide for reproductive assurance in terms of seed set, inbreeding depression is also possible in terms of offspring quality in *P. quinquefolius*. Evidence of inbreeding depression in *P. quinquefolius* despite high selfing rates is similar to that observed in *Aquilegia canadensis*, another forest perennial with small contemporary population sizes and a mixed mating system (Herlihy and Eckert, 2002, 2004). Though self-fertilization can achieve reproductive assurance when pollinators are scarce for *A. canadensis*, inbreeding depression limits the value of seeds and offspring produced this way (Herlihy and Eckert, 2002). For a long-lived species like *P. quinquefolius*, production of seeds by self-pollination may be a way to "wait out" adverse years when nonself pollen is limited. Such temporal variation has been a significant factor favoring the stabilization of mixed mating systems in evolutionary models (Goodwillie et al., 2005). If outcrossing opportunities are limited, self-pollination can be selectively favored, even if inbreeding depression in the offspring is considerable (Herlihy and Eckert, 2002).

Outcrossing pollination treatments—Differences in offspring quality between outcrossing within the population and with cultivated plants must be interpreted with care. In a different context, the increased leaf area and root biomass in the offspring of cultivated plants would suggest fitness benefits of outcrossing at this scale. However, in this context these differences likely reflect important genetic differences between cultivated and wild plants. The increased leaf area is largely due to the higher frequency of two-leaved individuals among offspring of Wisconsin-cultivated plants. Notably, previous demographic studies of *P. quinquefolius* in the wild have shown that plants reach the two-leaved stage after 3 yr or more of growth (Lewis and Zenger, 1982; Charron and Gagnon, 1991; Anderson et al., 1993; McGraw and Furedi, 2005). However, *P. quinquefolius* plants in cultivation after 3 yr

typically have three or more leaves and produce large amounts of seeds (Hughes and Proctor, 1981; Proctor and Bailey, 1987; Schluter and Punja, 2000; Hsu, 2002). Cultivated populations are propagated from seeds collected from plants prior to harvest, which can occur just after 2 yr of growth (Proctor and Bailey, 1987; Whitbread et al., 1996; Schluter and Punja, 2000; Hsu, 2002); this practice could putatively select for rapid achievement of large size and reproductive maturity. The techniques and environment of cultivation have been long recognized to change the life histories of formerly wild species (Darwin, 1882). Specifically in *P. quinquefolius*, humans may alter selection on size in the wild by preferentially removing large plants (Mooney and McGraw, 2007). Altogether, the cultivated–wild hybrids we observed resembled cultivated plants more than wild seedlings.

Previous studies in other plant species have also found increased performance of first generation intraspecific hybrids relative to parents (Hufford and Mazer, 2003). However, the fitness of hybrids can decrease in later generations, which is putatively due to the breakup of co-adapted gene complexes (Hufford and Mazer, 2003). In *Chamaecrista fasciculata*, for example, F1 hybrids had enhanced performance, but some F2 and F3 hybrids had decreased fitness relative to the parental population (Fenster and Galloway, 2000). Although these results suggest limitations to examining F1 fitness, the performance of cultivated–wild hybrids would determine whether future generations of hybridization are possible. Given the typical prereproductive period for *P. quinquefolius* of 5–10 yr, we cannot assess what fitness differences may occur in subsequent generations. As a long-lived species, F1 hybrids could presumably persist in populations of *P. quinquefolius* for some time.

Rapid growth of cultivated–wild hybrids may be achieved at a fitness cost under stressful natural conditions. Specifically, fewer plants produced by crosses with cultivated plants had mycorrhizal colonization relative to the offspring of crosses within the populations. Plants may be investing resources into leaf area and root biomass at the expense of allocations needed to recruit and sustain mycorrhizal fungi (Marschner, 2003). Early recruitment of mycorrhizal fungi may confer benefits later in life as resource demands increase or as soil nutrient pools are depleted (Treseder, 2004). One of the prominent features of cultivation of *P. quinquefolius* is the application of fungicides to prevent disease, insecticides to prevent herbivory, and fertilizers to compensate for inadequate soil nutrients (Proctor and Bailey, 1987). Although mycorrhizal associations occur in cultivation (Whitbread et al., 1996), high nutrient availability (specifically phosphorous) can decrease investment in mycorrhizal fungi by many species (reviewed in Treseder, 2004). Mycorrhizae may be less important in the cultivated environment than in low nutrient forest soils. Rapid growth could also occur at the expense of resistance to insect herbivory and fungal diseases. For example, ginsenosides—a class of saponins in *P. quinquefolius*—are important antifungal agents against ginseng-specific diseases (Nicol et al., 2002); but as with other carbon-rich defense compounds, ginsenosides would be produced at the expense of allocation to growth. The accelerated growth of cultivated–wild hybrids may have additional negative effects for longer periods through the cumulative effects of disease and environmental stress.

From our results, we see that crosses with cultivated plants confer ecologically important differences that appear to increase fitness, albeit early in the life cycle of first-generation hybrids. The success of our crosses demonstrates that there is no barrier at this stage to gene flow if cultivated individuals were introduced into wild populations (given that flowering times coincide). Though it may appear that we are detecting the relief of inbreeding depression, we should emphasize that such rapid growth has not been observed in our monitoring of thousands of plants over several years (E. H. Mooney and J. B. McGraw, unpublished data). Our results illustrate an important caveat to restoration using cultivated source material, even when no taxonomic distinction is made between wild and cultivated populations (Storfer, 1999; Hufford and Mazer, 2003; Tallmon et al., 2004). Similar issues have been addressed in the context of hybridization between wild and weedy species (Levin et al., 1996) and gene flow between wild and cultivated species (Ellstrand et al., 1999; Ellstrand, 2003). Gene flow can result in extinction through demographic swamping and genetic assimilation if hybrids outperform parental populations (Levin et al., 1996; Wolf et al., 2001; Haygood et al., 2003). This phenomenon has become increasingly important because of concerns about the escape of transgenes from genetically modified crops into wild or weedy relatives (Lu and Snow, 2005; Chapman and Burke, 2006). Recent application of genetic engineering to *P. quinquefolius* has produced plants that express antifungal proteins from *Oryza* species (Chen and Punja, 2004), adding to the potential consequences of outcrossing if transgenic plants become widely adopted.

General conclusions—Anthropogenic change is likely increasing the number of plant species worldwide that simultaneously experience small population size and gene flow from relatives in cultivation. This problem may be particularly relevant for medicinal plants of high value to humans that encourages both wild harvest and propagation. Small population sizes can limit recovery from harvest if pollen limitation or inbreeding depression reduces reproductive success. For plants like *P. quinquefolius* with a mixed mating system, self-pollination can provide reproductive assurance when pollen is scarce. However, even readily self-fertile species can have inbreeding depression, which can reduce offspring quality as we observed in *P. quinquefolius*. Evidence of inbreeding depression may not warrant the introduction of cultivated genotypes, given the possibility of introducing traits selected in cultivation. Our results from outcrosses with cultivated genotypes confirm that the precautionary principle should be applied when wild populations of rare species interact with their cultivated counterparts. If the accelerated growth of cultivated–wild hybrids persists to later generations, the integrity of populations in the wild could be threatened by well-intentioned restoration efforts. Overall, anthropogenic influences on population size in either direction can have far-reaching consequences for affected plant species.

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