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Limiting Factors of Five Rare Plant Species in Mesic Forests, Hawai'i Volcanoes National Park

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Technical Report HCSU-015

LIMITING FACTORS OF FIVE RARE PLANT SPECIES IN MESIC FORESTS, HAWAII VOLCANOES NATIONAL PARK

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ABSTRACT

Five rare or endangered plant species native to Kīpuka Puauulu and Kīpuka Kī were studied for two years to determine their stand structure, patterns of reproductive phenology, success of fruit production, potential pollinators, greenhouse seed germination rates, presence of soil seed banks, impacts of seed-predating rats, seed predation by insects, seedling predation by Kalij pheasant (*Lophura leucomelanos*), and seedling survival with different treatments. Species monitored were the trees *Hibiscadelphus giffardianus* (hau kuahiwi), *Melicope hawaiiensis* (manena), *M. zahlbruckneri* (alani), and *Zanthoxylum dipetalum* var. *dipetalum* (kāwa`u), and the vine *Sicyos macrophyllus* (ʻānunu).

Limiting factors identified for *H. giffardianus* were rat predation of seeds, bark-stripping, low fruit production likely resulting from the inter-relatedness of the planted population, and loss of original pollinators. For *M. hawaiiensis*, rat and native insect predation of seeds limited the number of seeds available, and natural seedling recruitment was very low. Pollination was effected by an alien insect species. For *M. zahlbruckneri*, native insect predation and rat depredation greatly reduced the number of seeds available for germination. The low proportion of flowers that became fruit indicated a lack of successful pollination or self-incompatibility. For *S. macrophyllus*, rat predation of seeds on the ground and in the seed bank reduced the number of seeds available for natural regeneration. Alien grasses were suspected to limit seedling recruitment. Most floral visitors were alien insect species. Seedling recruitment appeared to be the most vulnerable life stage for *Z. dipetalum* var. *dipetalum*. Both alien Kalij pheasants and unknown insect species were implicated as seedling predators/herbivores.

INTRODUCTION

Rare plant species comprise about 15% of the recorded flora of Hawai`i Volcanoes National Park (HAVO) (Higashino *et al.* 1988). Despite decades of protection from feral animals, reduction of invasive alien plants (Tunison and Stone 1992), and protection from wildfire, many rare plant populations have not increased or stabilized within the Park. Factors other than feral ungulates, alien plants, and fire are limiting recovery of endangered plant species of HAVO. Park managers are currently re-introducing or augmenting rare plant populations in selected Special Ecological Areas (SEAs) (Tunison unpublished, Tunison and Belfield unpublished). If these restored populations are to be self-sustaining, factors responsible for their original decline must be understood to allow development of adequate management strategies and to promote successful recovery of the rare species.

In HAVO, 25 plant species are listed as endangered or threatened, six are candidates for endangered status, and 29 are species of concern. Five of these rare plants are restricted within HAVO to mesic forest habitats, including Kīpuka Puaulu and Kīpuka Kī, two forests that have maintained high native plant diversity. Three of the rarities of mesic habitats are trees in the rue or citrus family (Rutaceae): *Melicope zahlbruckneri* (alani), a listed endangered species; *M. hawaiiensis* (manena); and *Zanthoxylum dipetalum* var. *dipetalum* (kāwa`u); the latter two are considered species of concern (U. S. Fish and Wildlife Service 2008). One endangered plant species, *Hibiscadelphus giffardianus* (hau kuahiwi) of the mallow family (Malvaceae), exists today only as plantings near its original habitat in Kīpuka Puaulu (Degener 1946). The fifth rare species treated in this study is *Sicyos macrophyllus* (`ānunu), a candidate endangered vine in the gourd family (Cucurbitaceae) found naturally in HAVO only within Kīpuka Kī and at one site in the Mauna Loa Strip.

The endangered alani is one of the rarest trees in the Hawaiian Islands. A Hawai`i Island endemic, *M. zahlbruckneri* is historically known from only three sites: Kīpuka Puaulu, Glenwood in the Puna District, and Moa`ula in Ka`ū. Currently, the tree is extant only in Kīpuka Puaulu and a recently discovered site at Laupāhoehoe Natural Area Reserve (U. S. Fish and Wildlife Service 1997a). Thirteen of the 17 *M. zahlbruckneri* specimens in the Bishop Museum Herbarium were collected at Kīpuka Puaulu, so the Park is clearly an important refuge for this imperiled species. In 1993-94, a project to determine the distribution and abundance of

rare plants in SEAs of HAVO (Stone unpublished) located 35 trees in Kīpuka Puauulu that were believed to represent the endangered *M. zahlbruckneri*; all except one were adult trees (Pratt and Abbott unpublished data). *Melicope zahlbruckneri* is a medium sized tree with very large opposite leaves, small inflorescences of unisexual flowers with white to pale green petals, and cuboid four-parted capsules. Male and female flowers are superficially similar and are borne on the same inflorescences. Detailed descriptions of the flowers of this and the other four study species are presented in Appendix I.

Melicope hawaiiensis is one of the most widespread species of the genus in Hawai`i; it is known from dry and mesic forests on Moloka`i, Lāna`i, Maui, and Hawai`i (Wagner *et al.* 1999). Despite its occurrence on multiple islands, the tree is nowhere common and has been collected on Hawai`i Island from relatively few scattered sites. The *M. hawaiiensis* populations of HAVO were formerly recognized as var. *gaudichaudii*, restricted to Kīpuka Puauulu and Mauna Loa upslope of Glenwood and Kīlauea, and var. *rubra* in Ka`ū and North Kona (Stone 1969); varieties are not currently recognized in this species (Wagner *et al.* 1999). In 1993-94, there were at least 150 *M. hawaiiensis* trees in Kīpuka Puauulu, including both adult trees and saplings (Pratt and Abbott unpublished data); subsequently a few trees were discovered on the boundary of the Park and Kapāpala Ranch near 1,070 m elevation (Thomas Belfield, pers. comm.). The tree is of medium to tall stature with smooth, pale brown bark, opposite narrowly-elliptic leaves, small unisexual flowers borne in few- to many-flowered inflorescences, and yellow-green, four-parted capsules densely covered with fine hairs.

Zanthoxylum dipetalum is distributed on the islands of Kaua`i, O`ahu, Moloka`i, and Hawai`i (Wagner *et al.* 1999), but is very rare except on Kaua`i, where most specimens have been collected (B. P. Bishop Museum website; National Museum of Natural History, Smithsonian Institution website). Rock (1913) recognized three varieties of *Zanthoxylum dipetalum*; he restricted *Z. dipetalum* var. *dipetalum* to Kaua`i and named the plant collected in Kīpuka Puauulu as var. *geminicarpum*. Subsequently, var. *geminicarpum* was sunk into var. *dipetalum*, which is currently considered a species of concern (U. S. Fish and Wildlife Service 2008). Apart from HAVO localities, the only Hawai`i Island site where var. *dipetalum* has been historically collected is Kawaihae Uka in Kohala (Hillebrand 1888); recent observations of the species have been made in Manukā Natural Area Reserve (Keali`i Bio, pers. comm.).

Within HAVO, *Z. dipetalum* var. *dipetalum* has been collected at Kīpuka Puaulu and within a small kīpuka between Kīpuka Puaulu and Kīpuka Kī in 1943 (Fosberg 1966). There is also a specimen collected at “Kīlauea near the lava tube” in 1934 (Morley 104-H, B. P. Bishop Museum Herbarium). Currently the species is restricted in HAVO to Kīpuka Puaulu, where 61 trees were mapped in 1993-94 (Pratt and Abbott unpublished data), and Kīpuka Kī, where more than 80 seedlings were planted in 2004 (Belfield *et al.* unpublished). This variety is a large dioecious tree with distinctive finely-fissured bark, dark green compound leaves with three to five leaflets and two reduced basal leaflets, waxy white to red two-parted flowers, and two-beaked follicular fruit (Wagner *et al.* 1999).

Hibiscadelphus giffardianus is endemic to the east slope of Mauna Loa on the island of Hawai`i and is a member of an endemic Hawaiian genus of seven species, which are all endangered or extinct (Wagner *et al.* 1999, Lorence and Wagner 1995). The species was discovered in 1911 in Kīpuka Puaulu (Rock 1913) before it became part of Hawai`i National Park, now HAVO. Only one tree was ever found; this individual was subsequently protected by a fence, and it provided propagation material until its death in 1930 (Degener 1946). Plants were grown from seeds and cuttings and were cultivated on private lands near the Park until the species was restored to Kīpuka Puaulu in the 1950s and 1960s (Baker and Allen 1977, Morris unpublished).

During the 1970s, hybrids between *H. giffardianus* and *H. hualalaiensis* were discovered in the Park after the two species had been planted in proximity to each other in Kīpuka Puaulu and seeds resulting from unassisted cross-pollination had been used to grow young plants for introduction to Kīpuka Kī. These natural hybrids were given the name *Hibiscadelphus x puakuahiwi* (Baker and Allen 1976a). The history of this hybridization and subsequent management decisions to remove hybrids were thoroughly discussed by Baker (1980) and Baker and Allen (1977). Recently, there have been approximately 300 plantings of *H. giffardianus* in Kīpuka Puaulu and Kīpuka Kī that were the progeny of 11 trees derived from the original tree. There remains (in 2009) one hybrid tree west of Kīpuka Kī, approximately a kilometer distant from *H. giffardianus* plantings. The current study monitored the remaining ca. 50-year old *H. giffardianus* trees and survivors of 205 seedlings planted in the two kīpuka in 1997, 2000, and 2001. *Hibiscadelphus giffardianus* is a medium-size tree with smooth bark, large round leaves with long petioles, large curved maroon to grey-green tubular flowers with

exserted stamens on a staminal column and a five-parted red stigma, and woody yellowish capsules covered with star-shaped hairs.

Sicyos macrophyllus is endemic to Hawai`i Island, where it ranges from Kohala Mountain in the north to windward Mauna Kea, Mauna Loa, and the saddle between the two large mountains (Wagner *et al.* 1999). The species is currently a candidate for listing as a threatened or endangered species (U. S. Fish and Wildlife Service 2008). The vine has also been known by the name *Sicyocarya macrophylla* (St. John 1978). Widely distributed on the island, the species has been recently observed at Pu`u Huluhulu in the saddle area and Hakalau Forest NWR, Kona Unit (Keali`i Bio pers. comm.). Historically, *S. macrophyllus* was collected by Joseph Rock in 1918 near the 23-mile marker of Highway 11 (specimen deposited in B. P. Bishop Museum Herbarium); the vine is apparently no longer extant on Kīlauea Volcano. The species was only recently discovered within Kīpuka Kī (1996-2000) and on the edge of a kīpuka in the Mauna Loa Strip (2005); it is not listed in earlier checklists of plants in HAVO (Higashino *et al.* 1988, Fosberg 1975, Fosberg 1966). *Sicyos macrophyllus* is a robust, fleshy vine with orbicular, palmately-lobed leaves. It has small, white unisexual flowers borne in separate inflorescences at the same node and dry, beaked fruit clustered together in a round head that appears as a spiny, pendent ball when mature.

The objectives of this study were 1) determine population size and stand structure for five rare plant species of mesic forests within HAVO; 2) measure flower and fruit production by monthly or bimonthly monitoring of phenology of selected populations; 3) investigate the cause of reproductive failure and lack of regeneration by following the fate of a sub-sample of flowers and fruit, identifying potential pollinators, quantifying seed germination rates and soil seed banks, and following seedling establishment; 4) evaluate the effect of alien plant cover in reducing seed germination and seedling recruitment; and 5) determine the role of rodents in flower, seed, and seedling predation.

THE STUDY AREA

Kīpuka Puaulu and Kīpuka Kī are islands of well-developed forest vegetation surrounded by more recent lava flows from Mauna Loa. Both are located on the lower east slope of Mauna Loa approximately 2.5 km northwest of Kīlauea Caldera within the section of the Park known as the Mauna Loa Strip, a corridor that connects the Kīlauea Caldera to the summit of Mauna Loa. Kīpuka Puaulu is at 1,200-1,280 m elevation adjacent to the Mauna Loa Road and extends north toward the Park boundary with Keauhou Ranch; the deep ash part of the kīpuka is approximately 100 ha. Kīpuka Kī is long and narrow, approximately 90 ha in size, at an elevation of 1,210 to 1,350 m; this kīpuka is bisected by the Mauna Loa Road (Fig. 1). The area within the two kīpuka has been reported as smaller than 90-100 ha by other authors (Mueller-Dombois and Lamoureux 1967); current area estimates are based on Geographic Information System (GIS) layers developed by HAVO and the U. S. Geological Survey. The two kīpuka are separated by a distance of about 0.8 km and a narrow arm of the Ke`āmoku Lava Flow. For one species, *Melicope hawaiiensis*, an additional site on the western Park boundary near 1,070 m elevation was visited and the small population there was monitored at the same time as Kīpuka Puaulu plants. The western site is within a small kīpuka east of Ka`ōiki Pali on the edge of the Ke`āmoku lava flow near the historical Peter Lee Road (Fig. 1); the kīpuka appears to be a remnant of a larger forest, but the portion within the Kapāpala Ranch has been altered and is now treeless pasture.

Geology and Soils

Soils of the study area are deep ash that has accumulated for thousands of years from volcanic activity of nearby Kīlauea Volcano. Detailed descriptions of soils in Kīpuka Puaulu and Kīpuka Kī are given by Mueller-Dombois and Lamoureux (1967), who dug soil pits to a depth of two meters and report soil depths of up to six meters in Kīpuka Puaulu. Previous soil surveys categorized the soils of Kīpuka Puaulu and Kīpuka Kī as Hanipoe silt loam, which is deep, dark brown to reddish brown, ash-derived soil with underlying layers of sandy loam, and Kekake extremely rocky muck, which is a thin organic soil over pāhoehoe bedrock (Sato *et al.* 1973). Substrates underlying the deep ash of the two kīpuka are greater than 4,000 years old (Lockwood *et al.* 1988). Lava flows surrounding the two kīpuka are late prehistoric Ke`āmoku `a`ā flows (Peterson 1967), a group of flows 100-750 years old (Lockwood *et al.* 1988).

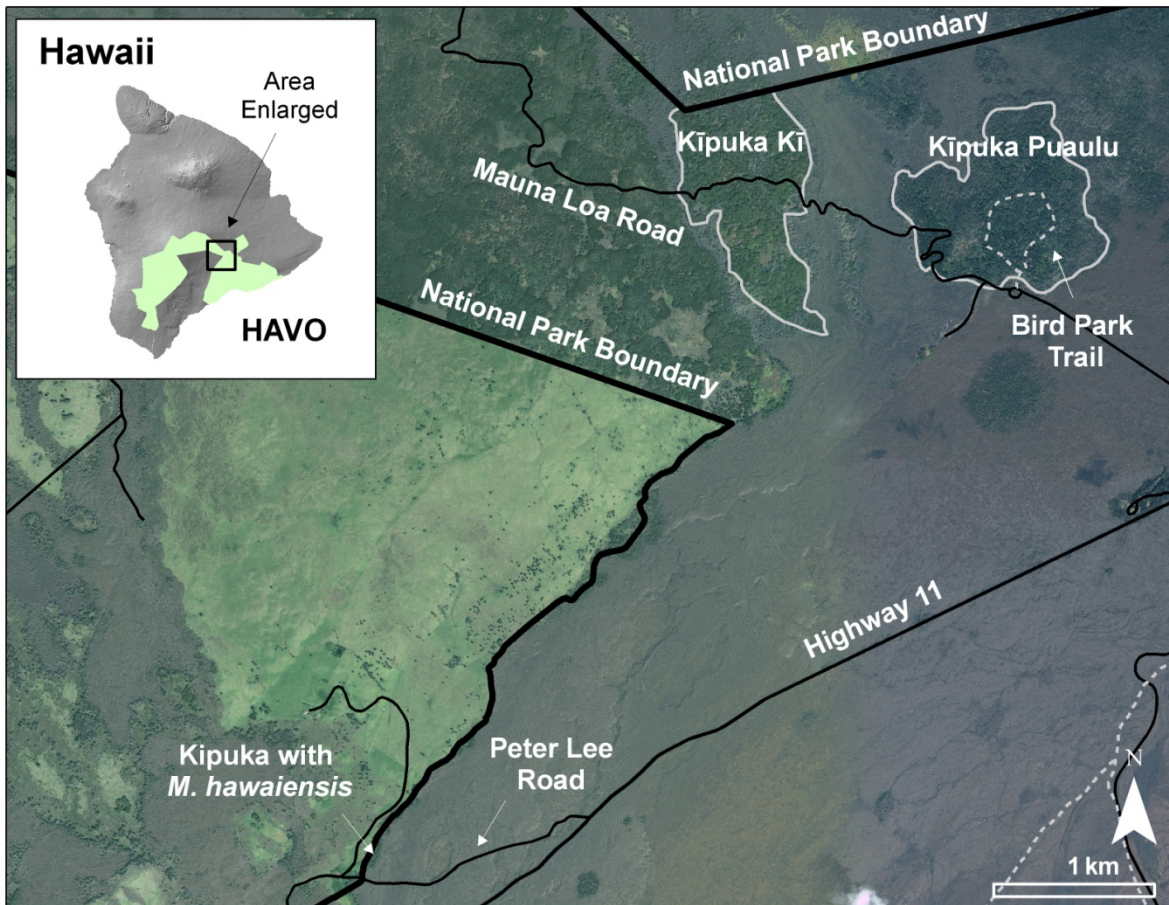


Figure 1. Location of the study sites within Hawai'i Volcanoes National Park (HAVO) including Kipuka Puauulu, Kipuka Kī, and a kipuka on the western Park boundary.

Climate

The climate of the two kīpuka and the surrounding lower slope of Mauna Loa is typically moist with dry summers (Doty and Mueller-Dombois 1966). The study sites are positioned below the trade wind inversion and receive moisture from clouds capped upslope at the inversion layer (Giambelluca and Schroeder 1998). The mean annual temperature ranges from 10 to 15.5° C (Hawai'i State Department of Land and Natural Resources 1970), and seasonal variation is relatively small with higher monthly temperatures during spring and summer and lower temperatures from October to May (Bridges and Carey 1973, 1974, 1975). Frost is a rare

occurrence on the lower slopes of Mauna Loa near 1,300 m elevation. Rainfall is associated with an orographic weather pattern, and mean annual precipitation is approximately 1,500 mm (Giambelluca and Schroeder 1998, Giambelluca *et al.* 1986). The driest months of the year are June to September, when monthly rainfall is typically less than 25 mm. By contrast, late fall, winter, and early spring months have higher rainfall averages that may exceed 100 mm (Bridges and Carey 1973, 1974, 1975).

Vegetation and History of Management

The lower Mauna Loa Strip is a transition zone between rain forests to the east and dry woodlands to the west and north. Natural vegetation of both Kīpuka Puaulu and Kīpuka Kī is a closed canopy, tall montane forest of *Acacia koa* (koa), *Metrosideros polymorpha* (‘ōhi‘a lehua), and *Sapindus saponaria* (mānele or soapberry) with an understory of native trees, such as *Coprosma rhynchocarpa* (pilo), *Pipturus albidus* (māmaki), *Myrsine lessertiana* (kōlea lau nui), *Psychotria hawaiiensis* var. *hillebrandii* (kōpiko `ula), *Pisonia brunoniana* (pāpala kēpau), *Nestegis sandwicensis* (olopua), and *Sophora chrysophylla* (māmane) (Gagné and Cuddihy 1999). Ground cover beneath native trees is composed of native herbs (*Peperomia* spp. or `ala`ala wainui) and terrestrial ferns, particularly *Microlepia strigosa* (palapalai), *Nephrolepis* spp. (kupukupu), and *Dryopteris* spp. (‘i`i and lau kahi). There are also many openings in the forest that are dominated by *Rubus argutus* (blackberry) and alien grasses. These grassy meadows are likely a legacy of past cattle grazing activities. Kīpuka Kī has a similar species composition, but the forest has a lower diversity of native tree species (Mueller-Dombois and Lamoureux 1967), and its understory is much less dense with unmanaged areas dominated by blackberry and alien pasture grasses.

The vegetation of the small kīpuka on the western boundary of the Park with a remnant population of *M. hawaiiensis* is tall *Metrosideros polymorpha* forest with an understory of *Sophora chrysophylla*, *Dodonaea viscosa* (‘a`ali`i), and *Osteomeles anthyllidifolia* (‘ūlei). Soils of the kīpuka are ash surrounded by `a`ā.

Prior to the addition of the Mauna Loa Strip to Hawai`i National Park in 1927, the area was part of a cattle ranch. Grazing ceased in the 1930s in Kīpuka Puaulu when a perimeter fence was constructed to exclude cattle. Grazing continued in Kīpuka Kī and the rest of the Mauna Loa Strip until 1948 (Apple 1954). Feral pigs (*Sus scrofa*) were fenced out of Kīpuka

Puauolu in the 1960s, and they were removed from Kīpuka Kī by 1989 (Katahira *et al.* 1993). Goats (*Capra hircus*) were recognized as threats to mesic forest vegetation in the 1970s (Spatz and Mueller-Dombois 1973), when they were excluded from parts of Kīpuka Puauolu (Baker and Reeser 1972). By 1980, fencing and removal efforts had excluded and cleared feral goats from Kīpuka Kī and the lower Mauna Loa Strip (Katahira and Stone 1982).

Kīpuka Puauolu was the first of the Park's 27 Special Ecological Areas to be managed to remove the most invasive alien plant species; systematic alien plant control began in 1985, when *Hedygium gardnerianum* (kāhili ginger), *Psidium cattleianum* (strawberry guava), *Solanum pseudocapsicum* (Jerusalem cherry), and *Tropaeolum majus* (nasturtium) were targeted (Tunison and Stone 1992). Alien plant control was extended to Kīpuka Kī in 1990 with experiments to find the most effective control measures for *Rubus argutus* and *Ehrharta stipoides* (meadow ricegrass); larger-scale efforts were planned to begin in 1993 (Tunison *et al.* unpublished). After a fire in woodlands adjacent to Kīpuka Puauolu, a suite of native trees and shrubs were planted in burned koa forest on the edge of the kīpuka (Loh *et al.* 2007). Subsequently, several grassland gaps on the edge and interior of Kīpuka Puauolu were treated with herbicide, and seedlings of native tree and shrub species were planted (Sierra McDaniel, pers. comm.). Alien plant control and native plant restoration continue in both kīpuka as part of a Park program to intensively manage SEAs (Rhonda Loh pers. comm.).

METHODS

Size Class Structure, Growth, and Mortality

All known trees of *Melicope hawaiiensis*, *M. zahlbruckneri*, and *Zanthoxylum dipetalum* var. *dipetalum* in Kīpuka Puaulu were visited in 2006 and 2008. A small disjunct group of *M. hawaiiensis* trees in a kīpuka on the western Park boundary was also visited. Diameters at breast height (dbh) were measured at 1.4 m, and heights of living trees in 2008 were recorded using a clinometer. These diameters and heights were compared with those measured on the same trees in 1993-94; some trees had heights estimated rather than measured 1993-94 (Pratt and Abbott unpublished data). Mortality over the 15-year period was determined by searching the mapped location; when the tree at that site was not found, the area was searched for fallen trunks and dead stumps. Trees for which no sign was found were considered dead. All 1997 and 2000-2001 plantings of *Hibiscadelphus giffardianus* in Kīpuka Puaulu and Kīpuka Kī, as well as seven older trees planted in 1951-1964, were visited in 2006 and 2008 and the height and dbh of each tree was measured. Heights and diameters of the 2000-2001 plantings were compared with measurements from 2000-2005 when plants were visited quarterly for three years and then on an annual or biannual basis through 2005 (Pratt unpublished data). All known sites supporting the vine *Sicyos macrophyllus* in Kīpuka Kī were visited in 2006. No previous data were available on population numbers or size classes for this species.

Sampling Methods for Phenology and Reproduction

Reproductive phenology was monitored bimonthly on randomly selected trees of *Hibiscadelphus giffardianus* (54 trees) and *Melicope hawaiiensis* (48 trees) for a period of approximately two years. The sample of *H. giffardianus* trees was visited monthly, starting in September 2007. Seven older trees from the original *H. giffardianus* plantings in 1951-1964 (Morris unpublished) were added to the monitored group in April 2007. All trees of *M. zahlbruckneri* were monitored; at first monitoring was bimonthly, but this changed to monthly visits from October 2007 through August 2008. After initial relocation of *Zanthoxylum dipetalum* var. *dipetalum*, a randomly selected group of 35 trees was monitored for six months, and then all 48 live trees and new finds were monitored bimonthly for another 1.5 years. For *Sicyos macrophyllus*, all four sites in Kīpuka Kī supporting the vine were visited monthly for two years, and observation points to estimate flower and fruit production were selected at random points around the perimeter of each patch of the vine (19 points total). Binoculars (8 x 32

Leica and/or 8.5 x 21 Pentax Papilio) were used to count the number of reproductive structures viewed at a pre-established direction from the observation point. Eighteen *S. macrophyllus* seedlings planted in Kīpuka Puaulu and Kīpuka Kī were monitored monthly for one year.

For *H. giffardianus*, large buds, flowers, young fruit, and mature brown fruit were counted on each monitored tree, and the number of small buds was estimated as 0, 1-5, 6-20, 21-100, or >100. Based on a small sample size of large buds followed daily (n = 5), large buds with an exposed corolla rounded at the tip became flowers within 2.8 days, so they were treated as flowers in analyses of reproductive phenology. Young fruit and mature fruit were grouped together for all analyses except that of rat exclusion trees, where they were evaluated separately. For five months in 2008 (February through June) we also counted the number of flowers that showed signs of nectar-robbing by native or alien birds. We quantified the proportion of open flowers with evidence of nectar-robbing by examining each flower with binoculars to look for the perforations and tearing of petals left by the birds' activities.

For *M. hawaiiensis* and *Z. dipetalum*, the number of inflorescences containing buds, open flowers, young green fruit, and mature brown fruit were estimated in categories of 0, 1-10, 11-50, 51-100, >100. *Melicope zahlbruckneri* immature green fruit and mature brown fruit were counted on each tree, and the number of inflorescences with buds or flowers was estimated using the same categories as were used for *M. hawaiiensis*. At *Sicyos macrophyllus* observation stations, inflorescences with female buds/flowers, male buds/flowers, small green fruit, large green fruit, and mature brown fruit were estimated in categories as were used for *M. hawaiiensis*. Binoculars were used for all phenology counts and estimates.

Success of Fruit Development

For all five species, plants sampled for monthly or bimonthly phenology were also selected for tagging of buds, flowers, or inflorescences. In *H. giffardianus*, all reachable branches (typically less than 10) were flagged and all buds, large buds, flowers, empty calyces, immature fruit, and mature fruit were counted. All accessible large buds, flowers, empty calyces, and immature (young) fruit were tagged with a colored wire or a numbered paper twist tie. The fates of tagged structures were followed at bimonthly samplings in 2006 and monthly visits in 2007-08.

Melicope hawaiiensis branches that were reachable had all female flowers and immature fruit tagged in 2006. In late 2007 through 2008, flower and fruit tagging was restricted to three trees (MH 89, 158, 159) with seven to nine reachable fertile branches. All inflorescences with female flowers and immature fruit were tagged and followed until they produce mature fruit or disappeared. On these same branches from three trees, bimonthly or monthly counts were made of all buds, flowers, immature fruit, and mature capsules. Because the *M. zahlbruckneri* population was small, all trees with reachable lower branches had female flowers and immature fruit tagged with paper twist ties and followed until maturity or disappearance.

Female *Z. dipetalum* var. *dipetalum* trees had inflorescences on accessible branches tagged in the same manner. Because entire inflorescences were tagged, we also followed the transition of buds to flower and immature green fruit. At the beginning of the study, the gender of trees was not always apparent, and some male flowers and buds were tagged. Once a tree was identified as male, its flowers were removed from the data set.

Sicyos macrophyllus female inflorescences were tagged in 2006 and 2007 at randomly selected observation points. These were followed monthly until mature dry fruit clusters were formed or inflorescences disappeared. The number of developing and aborted flowers and fruit were counted for each inflorescence.

Pollination Studies

Floral Visitor Observations

Composition of the floral visitor community and the rates of visitation were quantified by observing insect and bird activity at adjacent flowers of three plant species. Observations were made on dry days with sunny weather and light to moderate wind speeds. The identity of each floral visitor and the duration of the visit were recorded. Prior to the study, a species-level reference collection of insect visitors was made to facilitate identifications during the timed observations.

For *Hibiscadelphus giffardianus*, one to twenty-three flowers were observed for 20-minute periods between 0900 and 1500. Observations were made on 21 November 2006, 5 December 2006, 13 December 2006, 7 March 2008, and 1 April 2008, totaling 18.5 hours of observation. Flowers were observed at two distances: close range (one meter) for insects using

close-focusing 8.5x21 Pentax Papilio binoculars and far range (approximately 15 m) for birds using 8x32 Leica binoculars. For each floral visitor, the mode of entry into the corolla was also recorded.

For *Melicope hawaiiensis*, between two and 90 open flowers on an individual tree were observed simultaneously for 20-minute periods between 0900 and 1500. Observations were made on 20 September 2007, 6 March 2008, and 20 March, 2008 totaling 14 hours. For each floral visitor observed, the sex of the flower visited was also recorded.

At the largest group of *Sicyos macrophyllus* vines monitored, between three and 20 open flowers were observed in 10-minute periods between 0900 and 1500. Observations totaling 15 hours were made on 22 October 2006, 23 November 2006, and 30 November 2006. For each floral visitor, the sex of the flower visited was recorded.

Pollen Transport

To examine the extent to which floral visitors were capable of transporting *H. giffardianus* pollen, insects observed foraging on flowers on 30 June 2007 were collected and examined for presence of pollen (Kearns and Inouye 1993). Insects were first collected with either a butterfly net or captured directly into a 1-dram glass vial with a loosely closed top. The vial was then placed into a killing jar with a layer of plaster of Paris saturated with ethyl acetate. Once the insect was euthanized, the approximate amount and location of pollen on the insect's body was identified using a dissecting scope. To determine what percentage of the pollen observed was composed of *H. giffardianus* pollen, the insect was returned to its vial and washed by adding a 50:50 ethanol and water solution and shaken vigorously for two minutes (Krause and Wilson 1981). To ensure that most of the pollen was removed, the insect was withdrawn from the wash, allowed to dry, and then dabbed with a 1 mm³ section of hardened glycerin gel (Beattie 1971) under a dissecting microscope. The small cube of gel was added to the insect wash and centrifuged for four minutes at 12,000 rpm. The supernatant was then decanted and the remaining pellet mounted and sealed on a slide with a droplet of 50:50 glycerin and Calberla's stain solution. A reference collection of pollen from plants on the study site was used to identify pollen washed from insects.

Pollen Viability

A chemical test was used to estimate the viability of pollen within the *H. giffardianus* population. On 17 June 2008, 16 flowers with recently dehisced anthers were collected from 12 individual trees and brought into the laboratory for testing. A fine paintbrush was used to brush off a small amount of pollen onto a microscope slide. The pollen was stained with a droplet of MTT (2,5-diphenyl tetrazolium bromide), which tests for the presence of dehydrogenases in pollen as an indication of viability (Khatum and Flowers 1995). The droplet was allowed to dry and then a second application of the stain was applied. Once the second droplet dried, a droplet of glycerine and a cover slip were added to the pollen sample and sealed with nail polish. From a random field on the slide, 500 grains were counted and tallied as viable or nonviable. Viable pollen grains stain a dark brown while non-viable pollen remains colorless. As a control, a sample of pollen was killed with FAA (Formalin, acetic acid, and ethanol; 5:5:90, by volume) and then stained with a droplet of MTT to demonstrate the appearance of non-viable pollen to which viable pollen could be compared.

Stigma Receptivity

The degree to which viable pollen germinates on stigmas is sometimes used to determine stigmatic receptivity (Vaughton and Ramsey 1991). To determine the developmental stage at which *H. giffardianus* stigmas are most receptive, stigmas were examined for pollen germination following controlled pollination at various floral ages. In early June 2008, 48 mature buds were emasculated, randomly assigned a floral age to be cross-pollinated, and then enclosed in nylon bags with a fine mesh to be isolated from floral visitors. Floral ages assessed were the day of anthesis (n = 17), one day following anthesis (n = 14), and two days following anthesis (n = 17). Twelve trees were used for this experiment. Flowers were hand pollinated at the assigned age with fresh pollen from a flower of a different tree. Mesh bags were replaced immediately following pollination. After 24 hrs, flowers were collected and brought back to the lab.

To enable viewing of the stigmatic surface for the presence of germinated pollen, pistils were removed from flowers, and stained and viewed using fluorescence microscopy (Martin 1959). First, pistils were fixed in FAA for 24 hours, and then transferred to 70% ethanol for storage. Pistils were later softened in 8 N NaOH (sodium hydroxide) for eight hours, rinsed with

water, and then stained with a few drops of 0.0125% decolorized aniline blue in 0.067 N potassium phosphate buffer overnight. The decolorized aniline blue is absorbed by the callose in the cell walls of the pollen grains and pollen tubes and when viewed under a short wave light, they fluoresce. Pistils were then placed on a microscope slide with a drop of glycerol and then gently squashed with a cover slip and sealed with nail polish. Slides were viewed using a fluorescence microscope.

Pollen was deemed germinated if its pollen tube was longer than the length of the pollen's diameter. A stigma was considered receptive if at least one pollen grain germinated on the surface.

Pollen Limitation

For *H. giffardianus* and *S. macrophyllus*, fruit set was compared between naturally-pollinated flowers and flowers artificially supplemented with pollen by hand, to determine whether female reproductive success is limited by insufficient deposition of pollen on stigmas (Kephart 2005, Dafni *et al.* 2005). From March to May of 2007, *H. giffardianus* flowers were tagged as buds and randomly assigned to either control (no manipulation) or experimental (hand-pollinated) treatments. Upon anthesis, flowers in the experimental treatment were hand-pollinated using a fine paintbrush with an excess amount of fresh pollen from a flower of a different tree. In total, there were 155 control flowers and 148 flowers in the experimental treatment. After approximately one week, presence or absence of developing fruit for each flower was determined.

In November 2006, nearly 500 female inflorescences of *S. macrophyllus* were tagged as buds and randomly assigned to either open- or hand- pollinated treatments (n = 227 and 263 buds, respectively). Upon anthesis, flowers in the experimental treatment were hand-pollinated with fresh pollen gathered from a mix of donor plants. Because the sprawling growth pattern of *S. macrophyllus* vine made it difficult to differentiate between individual plants, an effort was made to pollinate flowers with pollen from flowers located at a distance > 3 m to the recipient flower. Pollen was applied by gently rubbing recently dehisced anthers across the stigmatic surfaces of the recipient inflorescence. After approximately one month, the number of mature green fruit was counted for each inflorescence. Fruit set and seed set are synonymous as each fruit contains only one seed.

Self-compatibility

The extent to which *H. giffardianus* flowers are self-compatible was examined by investigating the effect of different pollination treatments on pollen tube growth. Mature buds were tagged and randomly assigned to one of four treatments: (1) natural self-pollination (autogamy) – flower bagged with no manipulation (n = 14); (2) open pollination - flower left uncovered and no experimental manipulation (n = 6); (3) induced self-pollination (geitonogamy) – flower bagged and hand pollinated with pollen from its own anthers one day after anthesis (n = 11), and; (4) cross-pollination (xenogamy) – flower bagged, emasculated to prevent self-pollination, and hand- pollinated with fresh pollen (taken one day after anthesis) from another tree (n = 9). All flowers were collected two days following anthesis and examined for pollen tube growth. In the natural self-pollination and open-pollination treatments, each flower was emasculated (to prevent the flower's own pollen from being transferred onto the stigmas during transit) at time of collection and brought back to the laboratory where the total number of pollen grains on the stigmatic surfaces was counted under a dissecting microscope. Pollen tube growth was identified with the fluorescence microscopy technique used to examine stigma receptivity in all treatments.

Understory Thinning at Melicope zahlbruckneri

Because flower and fruit production was very low in the *M. zahlbruckneri* population, we reduced the dense understory cover surrounding selected small *M. zahlbruckneri* and followed the results to detect any change in reproduction of this tree. Five trees were randomly selected after excluding the several tall trees whose canopies extended far beyond the dense understory of trees in the kīpuka. In October 2007, the five selected trees (or groups of trees when several were adjacent) had the immediate understory surrounding the tree reduced by cutting all the *Coprosma rhynchocarpa* small trees and saplings in a radius of 2.5 m around each *M. zahlbruckneri* trunk. *Coprosma* trees were not killed; all stumps resprouted after cutting. Prior to understory thinning, cover-abundance estimates of all species in three height layers, as well as ground cover, were made using the Braun-Blanquet scale (Mueller-Dombois and Ellenberg 1974). The same size plots were used to estimate cover-abundance at five control trees without understory clearing. Subsequent to the clearing, production of flowers and fruit was compared between trees with understory clearance and those untreated.

Rat Exclusion Trees

To evaluate the impact of rats on flower and fruit production of *H. giffardianus*, rat predation was compared between trees from which rats were excluded and trees left unprotected. A random selection of *H. giffardianus* trees was made in both Kīpuka Puaulu and Kīpuka Kī using the criterion that they had to be free-standing and not touching any adjacent tree. Large trees dating from the 1951-1964 planting in Kīpuka Puaulu were not included in this analysis because they were closely surrounded by other trees and understory vegetation. These 26 exclusion trees had sheet metal flashing (51 cm tall) installed in a circle around the base of the tree to prevent rats from climbing the trunk. Adjacent trees without flashing were used as controls. Trees used in this experiment had the same data collected as other trees in the study; for more than two years the number of flowers (including large buds), empty calyces, young fruit, and mature fruit were counted, and rat predation was noted when present. Monitoring was bimonthly in 2006 and 2007 and then monthly from September 2007 until June 2008. Early in the monitoring period, we attempted to determine the movement of rats on trunks of a sample of 11 trees in each of the enclosure and control groups by the use of double rat tracking bands, with the lower band coated with fluorescent pink dye. Tracking bands were installed in both May and October 2006 and were left in place for three months.

To examine the impact of rats on young *Z. dipetalum* trees, 20 healthy plants were selected from survivors of 86 seedlings planted as part of HAVO restoration efforts in Kīpuka Kī in 2003 (Belfield *et al.* unpublished). Ten were randomly assigned to be caged to exclude rats and ten were left unprotected. In December 2006, cylindrical enclosures with tops were constructed of hardware cloth (with an opening diameter of 12 mm), placed around ten saplings, and securely staked to the ground to prevent rodent ingress. Cylinders were a minimum of 125 cm tall and were of a large-enough diameter so that leaves did not touch the wire. All 20 young plants were visited quarterly for 1.5 years to measure heights, count number of leaves, and determine mortality. Plants were also examined for rat depredation. We were provided with height data from the first two years after planting by Thomas Belfield of HAVO Natural Resources Management Division.

***Seed and Seedling Predation* Seed Plots in and Outside Rodent Enclosures**

Hibiscadelphus giffardianus seeds were planted in the Kīpuka Kī study site inside and outside two sets of rodent-proof enclosures and the resulting seedlings were monitored for 1.5 to two years. In January 2006, an equal number of seeds (17) were sowed in contact with soil in a grass-free area of 1 m² within a rat-proof enclosure and in an adjacent unprotected area of the same size, also without grass cover. Three replicates were placed in Kīpuka Kī adjacent to but not beneath *H. giffardianus* trees. Seeds within all treatment and control plots were covered by a light layer of leaf litter. Resulting seedlings were visited bimonthly or monthly, evaluated for mortality, and had their height measured for two years. A second set of three enclosures and controls was placed in the same area of Kīpuka Kī in February 2007. In this second trial, 59 seeds were sowed in contact with the soil in each of three 1 m² rat-proof enclosures and adjacent open plots. All enclosures and control plots were free of grass cover. Seedlings were monitored and measured bimonthly or monthly for 1.5 years.

Seed Predation by Rodents

Rodent predation on *H. giffardianus* seeds was tested by offering an equal number of fresh seeds (10) in three sets of plastic trays on the ground in the vicinity of plantings in both Kīpuka Puaulu and Kīpuka Kī. An equal number of seeds (10) was placed in an adjacent tray within a rodent-proof hardware cloth cage. Three replicate offerings were 20 m apart. For *M. hawaiiensis*, offerings of 10 fresh seeds were made in each of three pairs of adjacent open and closed rat bait stations; bait stations were used to exclude possible predation by Kalij pheasants. The bait stations were black plastic boxes (Protecta Brand) with an opening of 7 cm. Each replicate at a site near a fruiting *M. hawaiiensis* tree had one bait box intact and one box with the opening covered by screen to exclude rodents. *Melicope zahlbruckneri* did not produce enough seeds to use in a predation test.

Seed predation was tested twice for *Z. dipetalum*. In November 2006, three female *Z. dipetalum* trees bearing fruit were selected. Beneath each selected tree, an equal number (five) of ripe seeds was placed in a plastic tray open and available to predation and within a rat-proof box made of hardware cloth. In 2008, an equal number of seeds (eight) were offered in open and caged trays within Kalij pheasant enclosures at five *Z. dipetalum* trees. For *Sicyos macrophyllus*, whole fruit (either green or brown) were placed inside a rat bait station; an equal

number of fruit (10 in the first two trials and 20 in the third) was offered in a bait station with rodent-proof screen over the opening. Seed offerings were repeated in different seasons for *Z. dipetalum* and *S. macrophyllus*.

Seed Predation by Insects

When insect damage to *Melicope* capsules was observed, we identified the agent and quantified the percentage of capsules infested over a period of months. For *M. zahlbruckneri*, all mature capsules collected at eight monthly visits from December 2007 to September 2008 (for germination trials and propagation) were measured and examined for insect exit hole, frass, larvae, pupae, predated seeds, and whole seeds. For *M. hawaiiensis*, we systematically collected from three sample trees an equal number of mature or nearly mature capsules (15-30) three times between March and September 2008. We measured the capsules and examined them for insect infestation and predation as for the previous species. Mature seeds collected from these capsules were used in seed germination trials.

Seedling Predation by Kalij Pheasants (*Lophura leucomelanos*)

After natural and planted seedlings of *Z. dipetalum* var. *dipetalum* were observed with bite marks on leaves, we carried out two seeding trials to evaluate the impact of kalij pheasants on seedlings. In seeding trial one, we planted an equal number of seeds (22) in six grass-free plots of 1 m², three on both the north and south side of an open meadow in Kīpuka Puʻaʻulu. When likely Kalij predation was observed after nine months, we placed poultry netting around the three plots on the south side of the meadow and left the northern three unprotected. Poultry netting enclosures were not barriers to rodents, as they had small open gaps at the top and bottom. In seeding trial two, we planted 40 fresh seeds of *Z. dipetalum* var. *dipetalum* in each of six grass-free plots 1 m² in size; three of the plots were randomly assigned to be protected by poultry netting and three were left unprotected. All six plots were adjacent to each other and in the same area as half the plots in trial one, on the south side of the open meadow. For both seeding trials we counted the number of seedlings monthly, measured their heights, and recorded Kalij pheasant predation; plots were revisited a year after the second seed sowing.

When natural seedlings were noted beneath trees in summer 2008, we installed Kalij pheasant-proof fencing enclosures (1.5 m in diameter) beneath five heavily fruiting *Z. dipetalum*

var. *dipetalum* trees in June. One tree had no seedlings, but we anticipated their appearance because the tree bore a large crop of mature fruit. The tops of open exclosures were crossed with string to deter pheasants but allow fruit drop to enter exclosures. We monitored seedlings inside and outside these exclosures for eight months, measured seedling heights, and noted damage to seedlings attributable to Kalij pheasants.

Seed Plots and Seedlings with and without Grass

In May 2007, three pairs of seed plots 1 m² in size were established at randomly-selected points on the periphery of the largest natural group of *Sicyos macrophyllus* vines in Kīpuka Kī (Site A). Alien grass, primarily *Ehrharta stipoides*, was removed by hand from one plot in each pair, and the other plot was not manipulated. Seeds were peeled from the tough fruit coat and an equal number of seeds (35) was planted in each plot. Plots were visited monthly for 11 months to look for seedlings; a final visit was made 20 months after planting.

To test the impact of alien grasses on survival of seedlings, we planted an equal number of greenhouse-grown *Z. dipetalum* var. *dipetalum* seedlings (25) in a sunny, grassy meadow and in an adjacent grass-free area beneath native tree canopy in Kīpuka Puauulu. We visited seedlings quarterly for one year, recorded their survival, and measured their heights.

Seeds and Seed Germination

Soil Seed Bank

For all five target rare species the seed bank was examined two to three times over 2.5 years. *Hibiscadelphus giffardianus* trees were sampled in October 2006 and again during a 2.5-month period from February to May 2008. Each time a total of 15 trees was sampled with five soil cores; five trees in Kīpuka Kī and ten trees in Kīpuka Puauulu were sampled both times. The Kīpuka Puauulu trees included five large trees ca. 50 years old and five younger trees from the 2000-2001 planting. Five female trees of *Zanthoxylum dipetalum* bearing fruit were randomly selected for sampling in December 2007 and December 2008. At each tree, five soil cores were collected in a circle around the trunk at a distance of one meter (but still beneath the tree canopy).

For *Sicyos macrophyllus*, soil cores were taken beneath live vines at randomly selected observation points in June 2006, November 2007, and February 2009. In the first sampling, ten

cores were collected beneath the large vine designated A, and five each were collected from plants B and C. The second and third samplings collected five cores at each of the three natural plant groups (A, B, C). For *M. hawaiiensis*, ten soil cores were taken beneath each of three fruiting trees that were being monitored for reproductive success with tagged inflorescences. Coring was repeated in June and September 2008 and March 2009. The soil seed bank was investigated twice beneath three fruiting *M. zahlbruckneri* trees, when five soil cores were collected systematically around each tree one meter from the trunk, evenly spaced around the tree beneath the foliage canopy. For all species, the coring device was a bulb planter 10 cm long and 7 cm in diameter. Soils from the coring device were collected along with the litter overlying the core. Soil samples were air dried and sifted using soil screens with apertures smaller than the target seeds. All seeds whole or partial were retained and counted; rock, leaf litter, roots, and other structures were discarded. Whole seeds of the target species were planted in flats or pots in the HAVO greenhouse.

Seed Germination in the Greenhouse

Seeds of all five target species were collected from multiple plants several times throughout the study period. No more than 10% of the standing seed crop was collected. Seeds were removed from the fruit, cleaned, and air dried. Germination trials used fresh seeds whenever possible, but sometimes seeds were stored in a refrigerator for a month until a large enough sample of seeds could be collected. No special treatment was given to any seeds other than *Sicyos macrophyllus*, which in some trials were peeled out of the enclosing fruit wall or nicked using clippers. Seeds of all species were planted in the HAVO Natural Resources Management greenhouse in replicated flats filled with a potting medium of equal parts vermiculite, perlite, and cinder. Flats were misted with water three times a day. The time of first germination was noted, and counts of seedlings were made weekly. Except for *M. zahlbruckneri*, trials were terminated when seedlings ceased to appear or after one year with no germination. Some trials were terminated prematurely by rat predation of planted seeds.

For *Hibiscadelphus giffardianus*, there were four trials with three to seven flats per trial, using 25-58 seeds in each flat. Trials were initiated in May 2006 and in February, March, and August 2008; each trial was monitored for six to nine months.. *Melicope hawaiiensis* had three trials each with three to nine flats sowed with 10 to 72 seeds. The number of seeds available for germination trials varied greatly with the year; seeds were sowed in September 2006,

October 2007, and August 2008. Trials were terminated after 15 months; the final trial was transferred to HAVO propagation specialists after one year. *Melicope zahlbruckneri* seeds were scarce in 2007; the trial carried out that year used only three seeds in each of five replicate pots. A second trial in 2008 planted 43 fresh seeds in each of three replicate flats. The first trial was monitored for 18 months, and the second was followed for seven months when it was transferred to HAVO Plant Propagation Specialists.

Sicyos macrophyllus seeds were planted in six germination trials, each with three to five replicated flats. One trial in June 2006 used unpeeled seeds still enclosed in the inner wall of the fruit (utricle); two trials in March and June 2006 used seeds still inside the inner fruit wall nicked with clippers or partially peeled; and three trials in June 2007 used peeled seed with the fruit wall completely removed. The number of seeds used in each flat of five trials was 25, and one trial used 100 seeds per flat. Trials were monitored for 9 to 13 months. *Zanthoxylum dipetalum* var. *dipetalum* had three germination trials, each with three replicate flats in November 2006, July 2007, and February 2008. Seeds were collected from three to seven adult female trees during periods of heavy fruiting, and they were sowed immediately. The number of seeds per flat in the three trials was 30, 50, and 91, respectively. Trials were terminated after seven to twelve months, when seedlings stopped appearing. One group of *Z. dipetalum* seedlings was planted experimentally in Kipuka Puaulu, after following HAVO propagation sanitation protocols. All other seedlings resulting from germination trials were transferred to HAVO Natural Resources Management Division for restoration projects.

Seed Viability Tests

To complement germination trials and estimate seed viability, a tetrazolium test was used on 20 ungerminated seeds each of *Melicope hawaiiensis*, *M. zahlbruckneri*, *Zanthoxylum dipetalum* var. *dipetalum*, and *Sicyos macrophyllus*. For *Hibiscadelphus giffardianus* and its hybrid *H. x puakuahiwi*, only six to nine seeds were tested. Seeds were prepared by opening the seed coat and bisecting the endosperm and embryo with a razor blade. Prior to opening, the seeds of both *Melicope* species, *Z. dipetalum*, and *S. macrophyllus* were first imbibed in tap water for 24 hours. Each seed was individually treated with a 0.1% solution of 2,3,5-triphenyl tetrazolium chloride in a buffer of 66 mM KH₂PO₄ and 33 mM Na₂HPO₄ (pH ~7.0). Solution was added to completely cover both halves of the bisected seed. Hydrogen ions released as

products of respiration in a healthy embryo reduce tetrazolium chloride to form formazan, which appears red and stains healthy tissues (Baskin and Baskin 1998, Cottrell 1947).

Results of the tetrazolium tests were recorded approximately 20 hours after treatment. Each seed was individually inspected for staining. For the small *Melicope* and *Sicyos* seeds a dissecting microscope was used to locate and inspect the embryo. Viability was assessed by assigning each seed to a category representing the level of staining in the embryo (negative = no staining, weakly positive = embryo with barely detectable pink coloration, positive = embryo clearly pink, strongly positive = embryo with dark reddish staining).

Data Analysis

For all data sets except those collected during pollination studies, the majority of analyses were performed using two methods. For data recorded as categories, including phenology observations of structures that were too numerous to count efficiently and accurately (e.g., buds) and dichotomous variables such as survival, comparisons were made using chi-square tests of independence in contingency tables. For data that incorporated continuous variables, such as counts from phenology observations, growth, diameter, number of leaves, number of germinants, and frequency of events such as rat predation and nectar-robbing, means were compared using simple two-sample t-tests assuming equal variance. The t-tests were run using the Analysis ToolPak in Excel (Microsoft 2003).

Phenology patterns of *Hibiscadelphus giffardianus* were compared between locations and between rat enclosure treatments using a repeated measures analysis of variance (ANOVAR). This was run with SAS® 9.1 using the MIXED procedure (SAS Institute, Cary, NC). Repeated measures analysis was used to improve model accuracy by fitting a variance-covariance structure. The analysis was run with four different variance-covariance structures and then compared using AICC values. The results of the comparisons among Autoregressive(1) (AR1), Compound Symmetry (CS), Toeplitz (TOEP), and Unstructured (UN) indicated that UN was best fit in all cases, so all reported values come from output generated by models with UN specified as the variance-covariance structure. Denominator degrees of freedom were adjusted using the Kenward-Roger adjustment statement. For comparisons of bud production the analysis was run using raw category data. The flower and fruit data contained a large number of zeros associated with periods where some trees were not

producing, so count data was $\ln(\text{count}+1)$ transformed in order to meet the parametric assumptions of the ANOVA and to stabilize the error variance. Multiple comparisons were made using Tukey's adjustment to control experiment-wise Type I error rate.

In the pollination studies, mean visitation rates for each floral visitor species were calculated as the total number of flowers visited per total number of flowers observed per 10- or 20-minute observation period and then converted to the number of visits per flower per hour. The mean visitation rate was based on the average across all observation periods. Mean visit duration was calculated as the total time spent visiting a flower and averaged across individual visits independent of observation period. The two-tailed Mann-Whitney U test was used to compare mean visitation rates of floral visitors, and in the two monoecious species (*M. hawaiiensis* and *S. macrophyllus*), mean visit duration at male and female flowers.

The two-tailed Mann-Whitney U test was also used to compare the mean number of pollen grains washed off each insect species observed to visit flowers of *Hibiscadelphus giffardianus* and the average proportion of flowers within each inflorescence that developed green fruit in the *Sicyos macrophyllus* pollen limitation study.

For the natural self-pollination and open pollination treatments in evaluating self-incompatibility in *Hibiscadelphus giffardianus*, the total number of germinated and ungerminated pollen grains on the stigmatic surface was recorded. The number of bare stigmas without pollen was then compared between the two treatments and statistically evaluated using chi-square (χ^2) tests of independence in contingency tables. In the induced self-pollination and cross-pollination treatments, only pollen grains germinated out of 100 total grains in a random field of view were counted. The two-tailed Mann-Whitney U test was then used to compare the total number of germinated pollen grains between the two treatments. A pollen grain was considered germinated when the length of its tube was greater than the diameter of the pollen grain. Additional observations were made in all treatments for presence of pollen tube growth down the style entering the ovary. All statistical tests in the pollination studies were performed using SPSS 10.0 statistical package (SPSS Inc., Chicago, IL).

RESULTS

***Hibiscadelphus giffardianus*, Hau kuahiwi**

Growth and Mortality of Planted Population in Two Kīpuka

Hibiscadelphus giffardianus exists today in HAVO only as plantings in Kīpuka Puaulu and Kīpuka Kī. We monitored the survivors of 200 plantings made in 2000 and 2001, five trees planted in 1997, and seven large trees remaining from the first planting efforts with this species in 1951-64 (Morris unpublished) (Fig. 2). Growth and mortality data are presented here only for the 2000-2001 plants for which we have previous height and dbh data.

Survival of plantings was 73% with 146 of 200 trees alive in 2008. Mortality was higher in Kīpuka Puaulu (37%) than in Kīpuka Kī (17%). A similar pattern was seen in both 2000 and 2001 plants, with most losses occurring in the first two years after planting (Fig. 3). Growth was slow at first, but seedlings grew relatively quickly after the first year, and the mean height of both 2000 and 2001 plants was almost eight meters by 2008 (Fig. 4). There was no significant difference in the growth rate for height between the two kīpuka ($t = 0.3$, $df = 145$, $p = 0.79$) or the two plantings ($t = -0.1$, $df = 145$, $p = 0.92$). Overall growth rate was 0.9 (SD = 0.3, $n = 90$) m per year for 2000 plants and 0.9 (SD = 0.2, $n = 56$) m per year for 2001 plants. Mean growth rate of 0.9 m per year was the same in the two kīpuka (SD = 0.3, $n = 63$ in Kīpuka Puaulu; SD = 0.3, $n = 83$ in Kīpuka Kī).

Diameters increased steadily after planting. By 2003, the mean dbh of plants taller than 1.5 m was 1.8 cm (SD = 0.7, $n = 127$) with a significant difference ($t = -2.1$, $df = 126$, $p = 0.039$) between those of Kīpuka Puaulu (2.0 cm, SD = 0.8, $n = 52$) and Kīpuka Kī (1.8 cm, SD = 0.7, $n = 75$). By 2008, the group of trees measured in 2003 had increased in diameter to a mean of 6.0 cm (SD = 2.0, $n = 127$). The mean diameter of Kīpuka Puaulu trees in 2008 was significantly larger (6.7 cm, SD = 2.3, $n = 52$) than that of Kīpuka Kī trees (5.6 cm, SD = 1.6, $n = 75$) ($t = -3.2$, $df = 126$, $p = 0.002$).

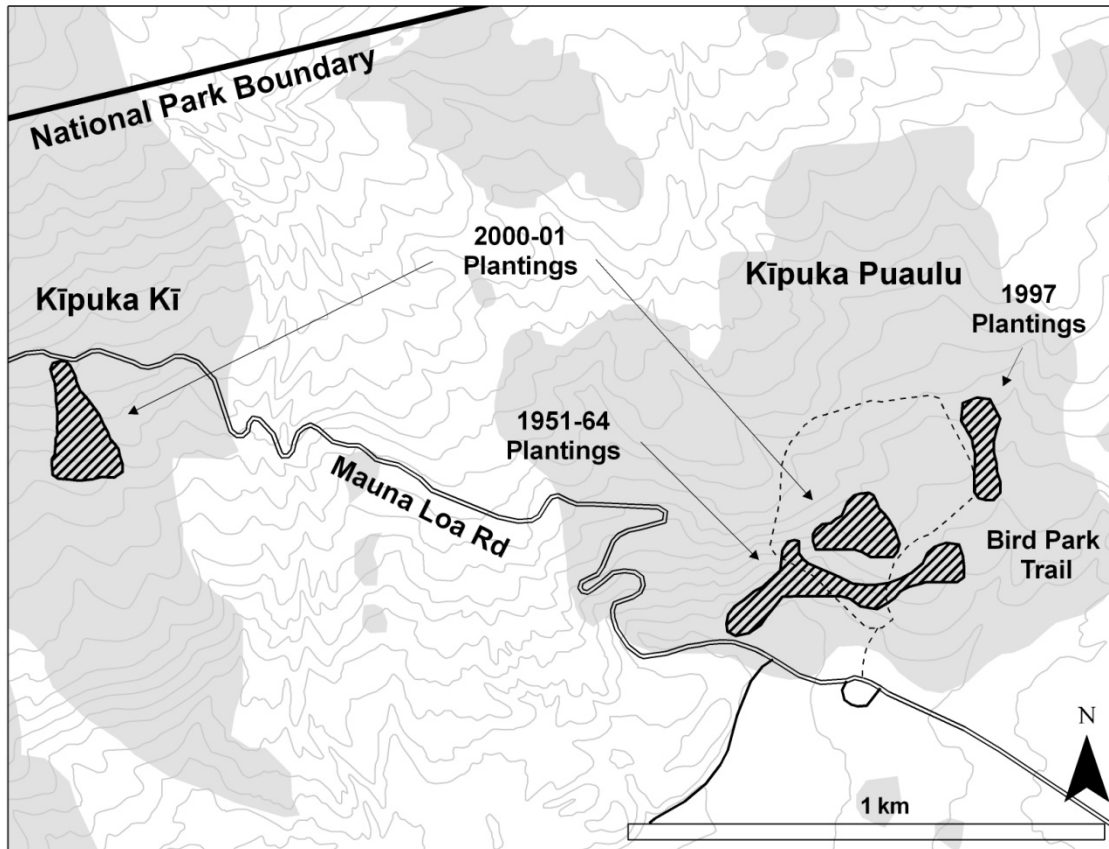


Figure 2. Areas with *Hibiscadelphus giffardianus* trees in Kīpuka Kī and Kīpuka Puaulu, HAVO, in 2008. Trees are survivors of plantings in 1951-1964, 1997, and 2000-2001.

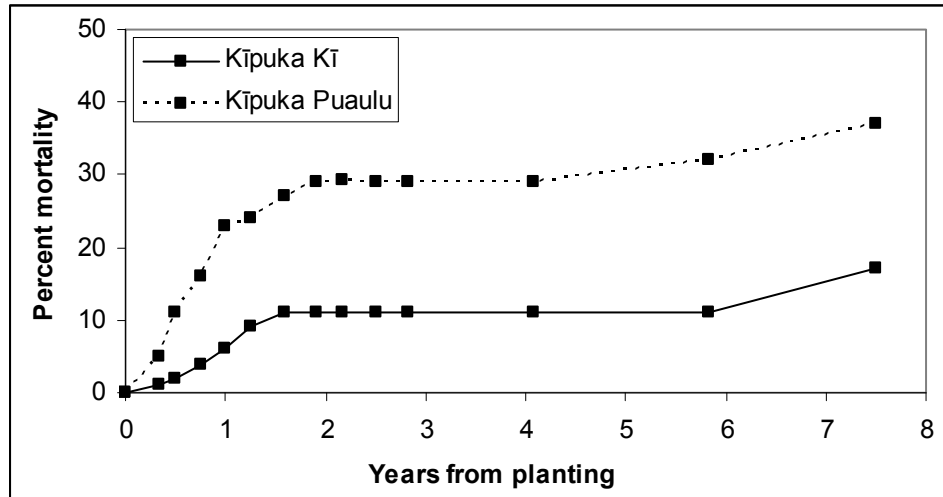


Figure 3. Mortality of *Hibiscadelphus giffardianus* planted in 2000 and 2001 in Kīpuka Puaulu and Kīpuka Kī.

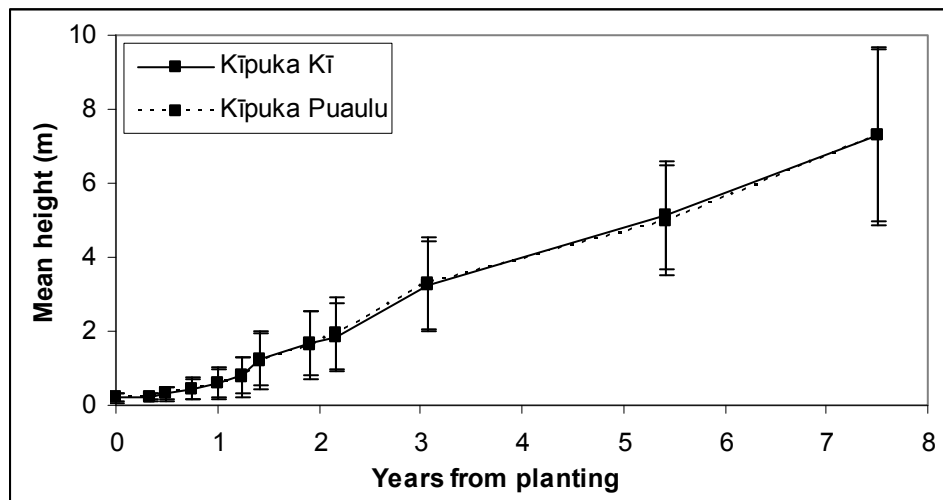


Figure 4. Mean (\pm SD) height of *Hibiscadelphus giffardianus* planted in 2000 and 2001 in Kīpuka Puaulu and Kīpuka Kī.

Reproductive Phenology 2006-2008

Over a two year period, >80% of monitored *H. giffardianus* trees from the 2000-2001 planting bore small flower buds throughout the year; only in summer 2006 did the percentage of trees with buds dip to 60% (Fig. 5). Flowers were produced by fewer trees than produced sma buds in all years monitored, and the pattern was more seasonal with summer peaks of flowering in May or June of three years. The year 2007 and the first half of 2008 had more trees producing flowers than did the second half of 2006. Fruit capsules were produced by fewer monitored trees than bore flowers, ranging from approximately 20 to 40% of trees (Fig. 5). A peak in trees bearing fruit was observed in winter 2007.

The mean number of flowers produced per tree per month for both kīpuka combined varied seasonally and annually (Fig. 6). More flowers were observed in spring and early summer, and a decrease in flower production was noted in the late summer and fall. The year from summer 2007 to 2008 appeared to be better for flower production than the previous year.

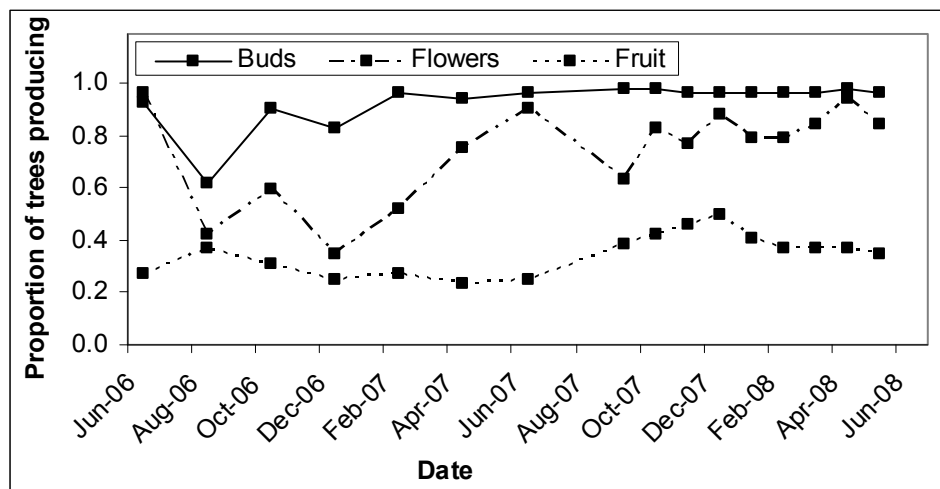


Figure 5. Proportion of *Hibiscadelphus giffardianus* trees producing small buds, flowers, and fruit (young and mature combined) in Kīpuka Puauulu and Kīpuka Kī, 2006-2008.

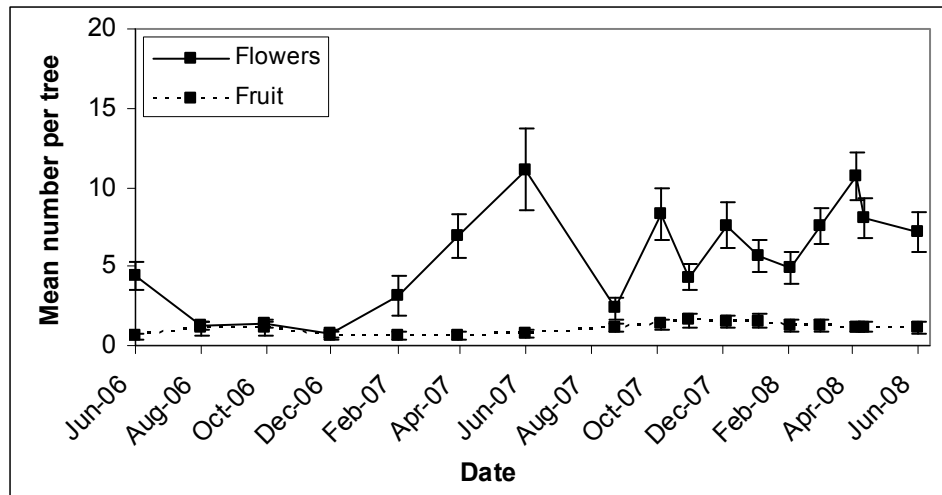


Figure 6. Mean number (\pm SD) of flowers and fruit on young *Hibiscadelphus giffardianus* trees in Kīpuka Puaulu and Kīpuka Kī combined, 2006-2008.

Low numbers of fruit were present in each month with small peaks of fruit production in the fall and winter months (Fig. 6). While fruit production was uniformly low in both Kīpuka Puaulu and Kīpuka Kī, the mean number of flowers was slightly higher during peak months in Kīpuka Puaulu. The number of flowers per tree in the spring peak (averaged over two years) was 12 in Kīpuka Puaulu and seven in Kīpuka Kī. Seasonality of flower production was similar in the two kīpuka.

The pattern of flower production in the seven large ca. 50-year-old trees was less seasonal than that seen in the younger plantings in Kīpuka Puaulu, and peaks were noted in several seasons, most notably in winter 2008. Fruit production in the older trees showed a rise during fall and winter similar to that seen in the younger trees. The average number of flowers and fruit observed on large trees was relatively high, with a peak of 30 flowers per tree in winter 2008 (Fig. 7). These older trees had larger crowns with many more branches than did trees planted seven to eight years ago, which probably contributed to their greater productivity.

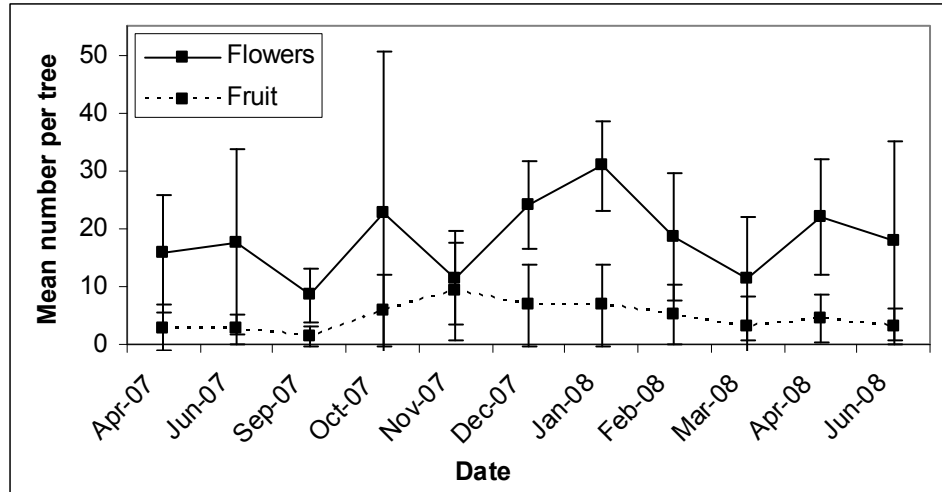


Figure 7. Mean number (\pm SD) of flowers and fruit on seven large (ca. 50 years old) *Hibiscadelphus giffardianus* trees in Kipuka Puauulu, 2007-2008.

Branch phenology. Counts of all buds, flowers, and fruit were made on selected lower branches of all monitored trees (young plants and >50-year-old trees) that had reachable fertile branches. The average number of buds per branch rose repeatedly during the two-year study in spring, fall and winter (Fig. 8). As might be expected, a higher number of buds was borne on the largest branches, and medium and small branches had proportionately fewer buds. The same pattern of larger branches being more productive was also seen in the mean number of flowers counted (Fig. 9). Seasonality of flower production was slightly later than bud production, with flower peaks in summer 2006 and winter 2007. The number of flowers detected at monitoring periods was much lower than the bud counts; even at flowering peaks, the number of flowers was typically only 10-20% the mean number of buds. The mean number of fruit capsules counted on branches was very low, but again large branches had a greater number of fruit than did smaller branches (Fig. 10). Seasonality of fruit was indicated with two peaks of fruit production in the fall and winter, just after the peaks of flower production.

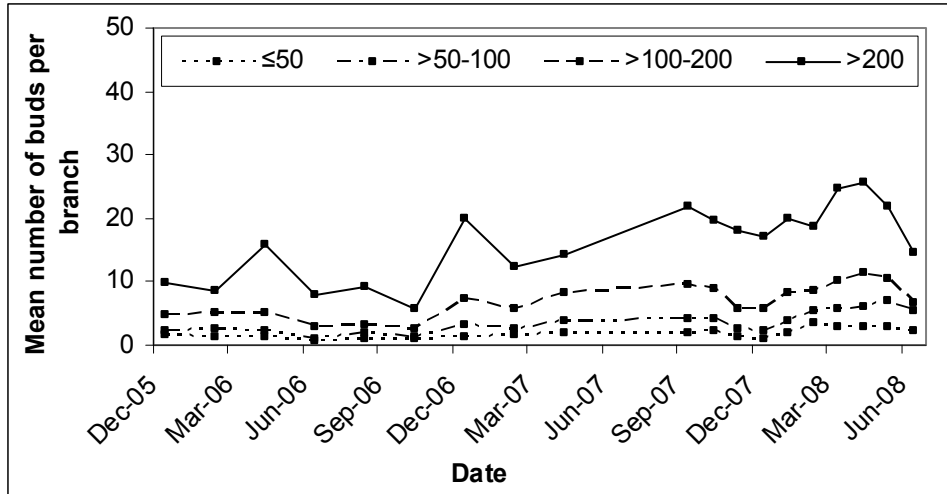


Figure 8. Mean number of small buds on different-sized branches of *Hibiscadelphus giffardianus* trees in Kipuka Puaulu and Kipuka Ki, 2006-2008. Branch lengths are ≤ 50 cm, $>50-100$ cm, $>100-200$ cm, and >200 cm.

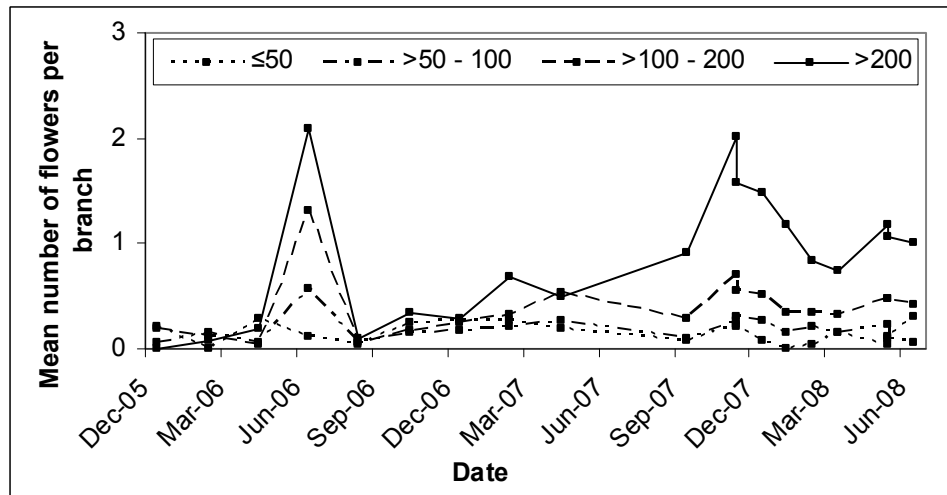


Figure 9. Mean number of flowers on different-sized branches of *Hibiscadelphus giffardianus* trees in Kipuka Puaulu and Kipuka Ki, 2006-2008. Branch lengths are ≤ 50 cm, $>50-100$ cm, $>100-200$ cm, and >200 cm.

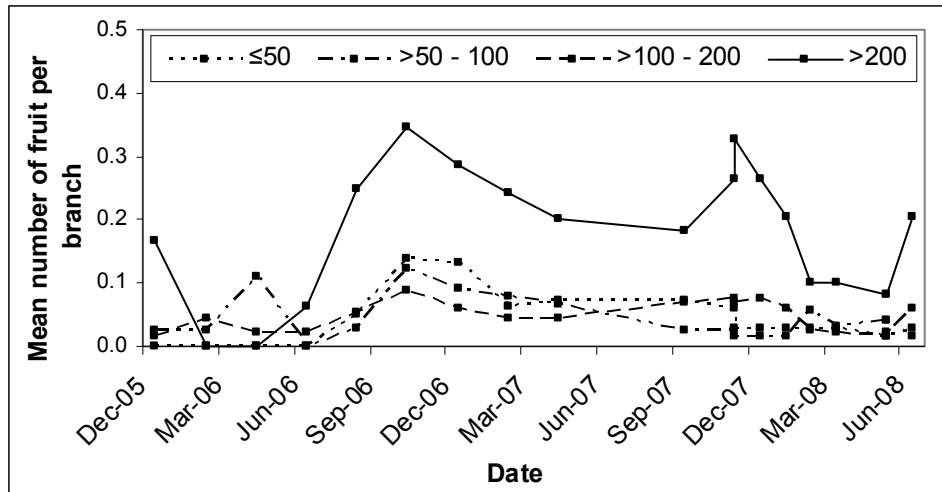


Figure 10. Mean number of fruit capsules on different-sized branches of *Hibiscadelphus giffardianus* trees in Kīpuka Puau and Kīpuka Kī, 2006-2008. Branch lengths are ≤50 cm, >50-100 cm, >100-200 cm, and >200 cm.

Success of Fruit Development

In 2007 and 2008, we tagged individual flowers and large buds with paper twist ties to determine the rate at which they became fruit. Of 788 tagged flowers, only 1.8% successfully formed fruit capsules. Mature fruit capsules were observed to persist on the tree up to six months. Based on seeds counted from capsules collected for germination trials, the mean number of seeds per capsule was 11.9 (SD = 3.2, n = 67). The monitored trees (planted in 2000-2001) produced an average of 1.7 fruit per year (SD = 2.9, n = 61), so the mean extrapolated number of seeds produced per tree per year was 20. The maximum number of fruit capsules produced yearly by any tree was 16; the total seed production of this heavy-bearing tree was estimated as 190.

Nectar-robbed Flowers

While monitoring monthly flower and fruit production and observing potential pollinators, we observed *Hibiscadelphus* flowers being visited by *Hemignathus virens virens* or Hawai'i Amakihi, and *Zosterops japonicus*, the Japanese White-eye. Most of these birds were nectar-robbing by thrusting their beaks into the base of the corolla from the side (see Pollination Studies section). In five monitoring periods combined (February-July 2008), 67% of

flowers showed signs of nectar-robbing (n = 1,738). Overall, the proportion of flowers showing damage was significantly greater in Kīpuka Kī (80%) than in Kīpuka Puaulu (65%) ($\chi^2 = 5.6$, df = 1, p = 0.02) (Fig. 11). Rates of nectar-robbing were higher in late winter and spring than in the summer monitoring periods.

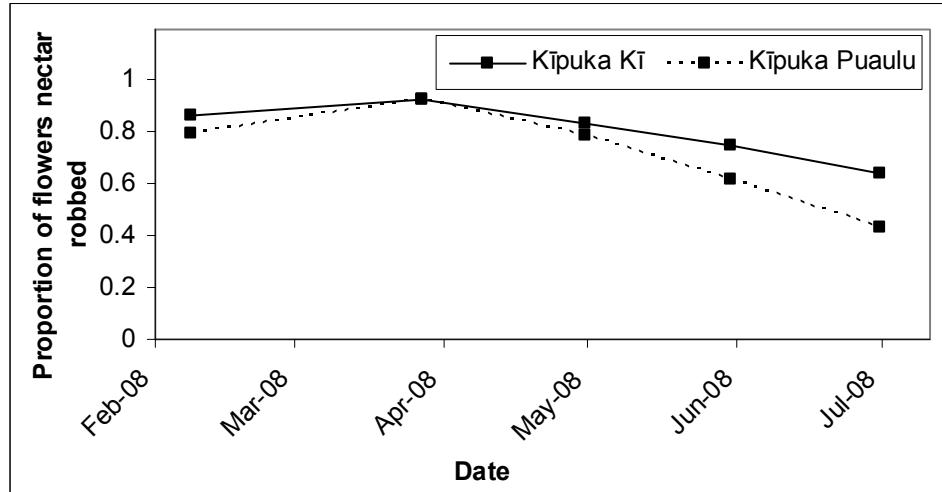


Figure 11. Proportion of *Hibiscadelphus giffardianus* flowers with evidence of nectar-robbing on trees in Kīpuka Puaulu and Kīpuka Kī, 2008.

Pollination Studies

Floral visitor observations - Birds. - During 18 hours of observation, birds visited *H. giffardianus* on 94 occasions. Sixty-one visits (64.9%) were of native *Hemignathus virens virens*, Hawai`i `Amakihi, and 33 (35.1%) were of non-native *Zosterops japonicus*, the Japanese White-eye. The frequency of visits did not differ significantly between Hawai`i `Amakihi (0.85 visits/flower/hr, SD = 0.41, n = 32) and Japanese White-eye (0.35 visits/flower/hr, SD = 0.80, n = 32) (Mann-Whitney U = 388.0, $n_1 = 32$, $n_2 = 32$, p = 0.05). On average, Hawai`i `Amakihi visitors spent 8.1 seconds (SD = 7.5, n = 61) at a single flower. This did not differ significantly from the mean visit duration of 8.4 seconds (SD = 8.5, n = 33) recorded for Japanese White-eye (Mann-Whitney U = 990.5, $n_1 = 61$, $n_2 = 33$, p = 0.90). Hawai`i `Amakihi exclusively robbed nectar from the base of flowers at each of the 61 visits

observed. In contrast, Japanese white-eye robbed nectar in 6% of the 33 visits observed and inserted their heads down into the opening of the corolla in the other 94% of visits. Outside of timed observation periods *Cardinalis cardinalis* the Northern Cardinal, a non-native seed-eater, was observed attempting to pry open two different seed capsules with no success.

Floral visitor observations - Insects – The fruit fly *Scaptomyza palmae* (Drosophilidae) was the most abundant visitor at *H. giffardianus* flowers comprising 21 of the 22 (95%) visits observed. A single visit from the honey bee *Apis mellifera* (Apidae) was the only other visitor detected during the observation periods. The mean visitation rate of fruit flies was 0.96 visits/flower/hour (SD = 1.32, n = 23). Duration of a single visit varied between 3 seconds to over 20 minutes, with an average duration of 779.1 seconds (SD = 507, n = 21). The fruit flies were observed on the interior and exterior of the corolla, but rarely contacted the anthers. The honey bee visited a single flower for 19 seconds and entered the apex of the corolla opening crawling down the length of the floral tube.

There were two other insect floral visitors of *H. giffardianus* documented outside of the observational period. *Vanessa tameamea* (Nymphalidae), the endemic Kamehameha butterfly, was occasionally observed visiting flowers extracting nectar both legitimately at the apex of the corolla and illegitimately through holes chewed through the floral tissue by rats (*Rattus* spp.). A native sap beetle, *Prosopius subaeneus* (Nitidulidae), was present within the corolla tube of each flower examined.

Pollen transport. Insects collected for pollen identification consisted of 18 *Scaptomyza palmae* fruit flies, 14 *Prosopius subaeneus* sap beetles, and one *Apis mellifera* honey bee. Before insects were washed, visual examination of the insect integument identified three fruit flies, one sap beetle, and one honey bee carrying pollen. Total pollen grains were distributed as follows: one on the wing and two on the tarsi of the fruit flies, one on the tarsus of the sap beetle, and 50 on the corbiculae (pollen baskets on the hind tibiae) of the honey bee.

Insect washing identified a greater number of individuals of fruit flies and sap beetles carrying pollen than were observed during the visual examination (Table 1). The average number of *H. giffardianus* pollen grains removed from washed insects did not differ significantly between fruit flies (0.11 pollen grains, SD = 0.32, n = 18) and sap beetles (0.29 pollen grains, SD = 0.61, n = 14) (Mann-Whitney U = 112.0, $n_1 = 18$, $n_2 = 14$, $p = 0.61$). The single honey

bee collected had no *H. giffardianus* pollen and 25 grains of pollen from other plant species. A significant difference was found between the average number of *H. giffardianus* pollen and the average number of pollen from species other than *H. giffardianus* for both fruit flies (Mann-Whitney U = 86.0, $n_1 = 18$, $n_2 = 18$, $p = 0.004$) and sap beetles (Mann-Whitney U = 55.5, $n_1 = 14$, $n_2 = 14$, $p = 0.03$). Proportionally, *H. giffardianus* pollen made up 11.7% of the average pollen load for fruit flies and 11.9% of pollen on sap beetles.

Table 1. Pollen load composition of insects collected on flowers of *Hibiscadelphus giffardianus* on June 30, 2007.

Insect type	Total No. of individuals	No. of individuals with pollen	Average no. of pollen grains per individual (SD)	
			<i>Hibiscadelphus giffardianus</i>	Other plant species
Fruit fly (<i>Scaptomyza palmae</i>)	18	3	0.11 (0.32)	0.83 (0.92)
Sap beetle (<i>Prosopeus subaeneus</i>)	14	3	0.29 (0.61)	2.14 (3.03)
Honey bee (<i>Apis mellifera</i>)	1	1	0	25

Pollen viability and stigma receptivity. At anther dehiscence, 75.4% of the pollen tested was viable. Stigmas were 100% receptive at each stage of flower development tested.

Pollen limitation and self-compatibility. Pollen limitation could not be evaluated because there were no developing fruit detected in any of the hand- or open-pollinated flowers. All flowers wilted and abscised at the petiole three to five days after anthesis.

The percentage of flowers observed to have stigmas with pollen differed significantly between the naturally self-pollinated and open pollination treatments ($\chi^2 = 8.94$, $df = 1$, $p = 0.003$) (Table 2). There were no pollen grains observed on the surface of stigmas in the naturally self-pollinated treatment versus an average of 9.0 (SD = 10.4, $n = 6$) in the open-pollinated flowers. Cross-pollinated flowers had significantly greater number of germinated pollen grains

on the stigma than the induced self-pollination treatment (Mann-Whitney $U = 10.5$, $n_1 = 9$, $n_2 = 11$, $p = 0.003$). The mean proportion of germinated pollen grains varied from 0 in the naturally self-pollinated treatment to 63% in the open pollination treatment, 33% in the induced self-pollinated treatment, and 61% within the cross-pollinated treatment. Pollen tubes were not detected in the styles of flowers in any of the treatments. Pollen grains developed more than one pollen tube per grain (polysiphonous condition), but tubes did not penetrate the surface of the stigma.

Table 2. Number of *Hibiscadelphus giffardianus* flowers in each pollination treatment and the resulting pollen deposition and germination on the stigmatic surface.

Treatment	No. of flowers	No. of flowers with pollen on the stigma	Mean no. of pollen grains per stigma (SD)	Mean no. of germinated pollen grains per stigma (SD)
Natural self-pollination	14	0	0.0	0.0
Open-pollination	6	3	9.0 (10.4)	3.7 (6.2)
Induced self-pollination	11	11	Many ^a	32.6 ^b (18.0)
Cross-pollination	9	9	Many ^a	60.8 ^b (12.9)

^a Copious amounts of pollen were applied to stigmas of hand-pollinated flowers.

^b Values represent the number of germinated pollen grains of a 100 total in a random field of view.

Rat Exclusion Trees and Controls

In a comparison of the flower and fruit production of young *H. giffardianus* trees protected by a rodent barrier and those unprotected, there was a significant difference in the number of flowers produced on rat-excluded trees versus control trees ($F_{1,50} = 4.7$, $p < 0.0001$). The mean number of flowers was considerably greater among control trees (7.3, SD = 11.1, $n = 464$) than on enclosure trees (3.3, SD = 5.8, $n = 368$). A similar pattern was seen with empty calyces, a transition state between flower and immature fruit. The mean number of empty calyces was 0.3 (SD = 0.8, $n = 368$) on enclosure trees and 0.6 (SD = 1.5, $n = 464$) on control trees ($F_{1,50} = 4.4$, $p < 0.04$). No significant difference was detected in the means of young or mature fruit compared between enclosures and control trees (young fruit $F_{1,50} = 2.1$, $p = 0.16$; mature fruit $F_{1,50} = 0.4$, $p = 0.53$).

When all trees, regardless of enclosures, were compared between the two kīpuka, the number of flowers was significantly higher in Kīpuka Puaulu (6.5, SD = 8.9, $n = 256$) than in Kīpuka Kī (5.1, SD = 9.5, $n = 576$) ($F_{1,50} = 21.6$, $p < 0.0001$). There was no significant difference in empty calyces, young fruit, or mature fruit in the two kīpuka. In a pairwise comparison of enclosure trees in Kīpuka Puaulu and Kīpuka Kī, there was no significant difference in the mean number of either young fruit ($t = -0.4$, $df = 48$, $p = 0.9826$) or mature fruit ($t = -1.1$, $df = 48$, $p = 0.6671$), although Kīpuka Puaulu had slightly larger means for both young fruit (0.3, SD = 0.7, $n = 96$) and mature fruit (1.0, SD = 1.2, $n = 96$) than did Kīpuka Kī (young fruit 0.2, SD = 0.6, $n = 272$ and mature fruit 0.8, SD = 2.0, $n = 304$).

We noted two episodes of intensive bark stripping by rats over the course of the monitoring. In April 2007, 12 of the unprotected *H. giffardianus* trees had severe bark stripping with a mean number of 16 branches stripped of bark per tree. The April 2008 stripping episode was less severe, with five of 30 trees showing signs of rat predation on an average of five branches per tree. No bark stripping was seen in enclosure trees, except for two that were compromised by the growth of adjacent *Pipturus albidus* shrubs, which had branches in contact with *H. giffardianus* trees above the enclosures.

Rat-tracking bands. In bands installed in May 2006 and left in place until July, we detected rat tracks on two unprotected trees: one each in Kīpuka Puaulu and Kīpuka Kī. Both

trees were later observed with bark stripping damage. We repeated the tracking band installation in October 2006 through December, when no rat tracks were observed.

Predation of flowers and fruit. Between June 2007 and June 2008, we recorded all rat-predated buds, flowers, and fruit all on monitored trees including the seven older plantings. Over the year, there were 41 small buds, 26 large buds, and 57 flowers that exhibited signs of rat chewing. During the same period, 10% of all 203 fruit observed were damaged or predated by rats. Most of these predated fruit were mature (14) rather than immature developing fruit (six). Rat predation of buds, flowers, and fruit was noted on 14 trees during this year, six of them larger trees dating from the 1951-1964 planting. All but two trees exhibiting signs of rat-predation were unprotected trees; the two enclosure trees with predated flowers and fruit had been compromised by the growth of adjacent *P. albidus* branches, allowing rats to move onto the tree without ascending the trunk. There was a significantly higher percentage of flower predation on control trees in Kīpuka Puaulu (4%, $n = 1,935$) than in Kīpuka Kī (<1%, $n = 790$) ($\chi^2 = 509.8$, $df = 1$, $p < 0.001$).

Seed and Seedling Predation

Seed plots inside and outside rodent enclosures. In the first set of grass-free rodent enclosures and controls in which *H. giffardianus* seeds were sowed in contact with the soil (to simulate natural seed fall), germination was observed in April, three months after sowing. After two years, a total of six seeds had germinated, five in caged plots (10%, $n = 51$) and one in open plots (2%, $n = 51$). There was no statistical difference between enclosures and control plots ($\chi^2 = 2.9$, $df = 2$, $p = 0.24$). Over the two-year period, enclosure plants had a mortality of 20%, and the open plot seedling persisted. There was no observed loss or damage due to rodents. Mean heights of enclosure seedlings progressed steadily and after 2.5 years, survivors were approximately two m tall; the seedling in the open control plot remained small.

In the second seeding experiment inside and outside rodent enclosures in February 2007 germination was noted within 1.5 months. A total of nine seeds germinated in this second seeding; three seedlings appeared in enclosures (1.7%, $n = 177$) and six in open control plots (3.4%, $n = 177$). There was no significant difference in the number of seedlings within enclosures and control plots ($\chi^2 = 0.4$, $df = 2$, $p = 0.80$). Over more than a year of monitoring, there was a mortality of 67% in caged seedlings and a loss of 50% in control plots; no loss due

to rodents was observed. The height of the single remaining enclosure seedling was greater than 50 cm, while control plot seedlings achieved a mean height of 30.8 cm (SD = 19.2, n = 4) after more than a year.

Seed predation by rodents. In October 2006, we offered ten fresh *Hibiscadelphus giffardianus* seeds in open trays at three sites separated by 20 m in both Kīpuka Puaulu and Kīpuka Kī. A control tray with ten seeds within a rodent-proof cage of hardware cloth was placed adjacent to each open tray. Within two days, two of three open trays in Kīpuka Puaulu had all seeds consumed, and a pile of seeds husks beside the trays indicated the seed predator was a rodent rather than a bird. Within five days all seeds in open trays were consumed in Kīpuka Puaulu, and none was disturbed in the caged trays. In Kīpuka Kī, one open tray was disturbed within seven days of the offering, with eight seeds chewed or missing. A second open tray was disturbed after 25 days with six seeds missing and three chewed. After 31 days, two of three open trays had predated seeds (67% of seeds); the third open tray and the control trays in cages were undisturbed.

Seeds and Seed Germination

Soil seed bank. The soil seed bank beneath *H. giffardianus* trees was examined in October 2006 and February/May 2008. No *H. giffardianus* seeds were detected in soil samples collected in 2006. In 2008 samples, there was one intact *H. giffardianus* seed recovered from soil cores of one tree in Kīpuka Kī and eight partial seeds from one tree in Kīpuka Puaulu. The intact seed was sowed in a greenhouse pot but did not germinate. The partial seeds had been predated by rodents leaving only fragments of the seed coat. Only 1.3% of soil cores contained an identifiable whole seed of the target rare species. By contrast, soil cores contained many whole seeds of the forest dominants *A. koa* and *S. saponaria* and a few seeds and fruit of common forest trees such as *C. rhynchoarpa*, *M. polymorpha* and *M. lessertiana*.

Seed germination in the greenhouse. There were four successful germination trials with *Hibiscadelphus giffardianus* in the HAVO greenhouse. Germination was first observed in flats within seven to twenty days, and germination in some trials continued for more than six months (Appendix II, Table 1). Germination rates were somewhat variable. The mean in the 2006 trial with the most replicate flats was the highest at 30.9%, followed by a rate of 25.6% in the trial begun in March 2008. The trial sowed in February 2008 had the lowest average germination

rate of 9.0%. The final trial initiated in August 2008 had an average germination of 19.5%, and seedlings continued to appear until January 2009.

Seed viability tests. The tested *H. giffardianus* seeds had been stored at 3 C° for one month. Upon opening the seed coat the cotyledons and embryo were clearly visible. All seeds appeared to be well-developed and healthy. Of the six seeds tested two were strongly positive and the remaining four were recorded as positive.

Two collections of seeds from one hybrid *H. x puakuahiwi* tree were tested. The first collection was seed that had been stored for eight months at 3 C°. When the seed coats were opened, all of the seeds appeared partially empty due to desiccation of the endosperm and cotyledons. The embryos, however, seemed healthy. The second collection of seeds was obtained fresh as young fruit. The fruit capsules were allowed to dry and open at room temperature. When cut open, the fresh seeds were mostly empty, had reduced endosperm and cotyledons, and appeared to be infected with a fungus growing on the inside of the seed coat wall. Some seeds appeared dry and desiccated, while others were partially decomposed with a residue of mucus. Despite the poor appearance of the seeds, embryos seemed intact but dry in the desiccated seeds and covered by viscous fluid from the deteriorating endosperm in the others.

Despite the unusual appearance and longer storage period of the first round of hybrid seed, the results were 100% (n = 11) strongly positive. Of nine seeds tested in the second round four had strongly positive results, four had positives, and one had a weakly positive result.

***Melicope hawaiiensis*, Manena**

Size Class Structure, Growth, and Mortality

The number of living trees found in Kīpuka Puaulu (Fig. 12) and a kīpuka on the western Park boundary in 2008 (Fig. 13) was 114 (including recruitment and trees missed in 1994). During rare plant surveys of HAVO SEAs in 1993-94, 150 *Melicope hawaiiensis* trees were mapped in Kīpuka Puaulu, and none was found in Kīpuka Kī (Pratt and Abbott unpublished data). The small population in the kīpuka on the western boundary had not yet been found in 1994. The

size class structure of the population in Kīpuka Puaulu was U-shaped in 1994, with the addition of a few very large trees in the highest diameter class (Fig. 14). The presence of 50 small (and presumably young) trees with diameters <2 cm, as well as almost 30 trees 2-5 cm in diameter, indicated that natural reproduction was occurring within the population. By 2008, the population structure of *M. hawaiiensis* had changed dramatically. Lower size classes were much reduced from those of the earlier period, and almost half of the trees >20 cm in diameter had been lost during the interval. The size class distribution in 2008 was relatively flat with similar numbers of trees in the smaller two classes and fewer trees in the three upper diameter classes. No trees >40 cm remained in 2008 (Fig. 14).

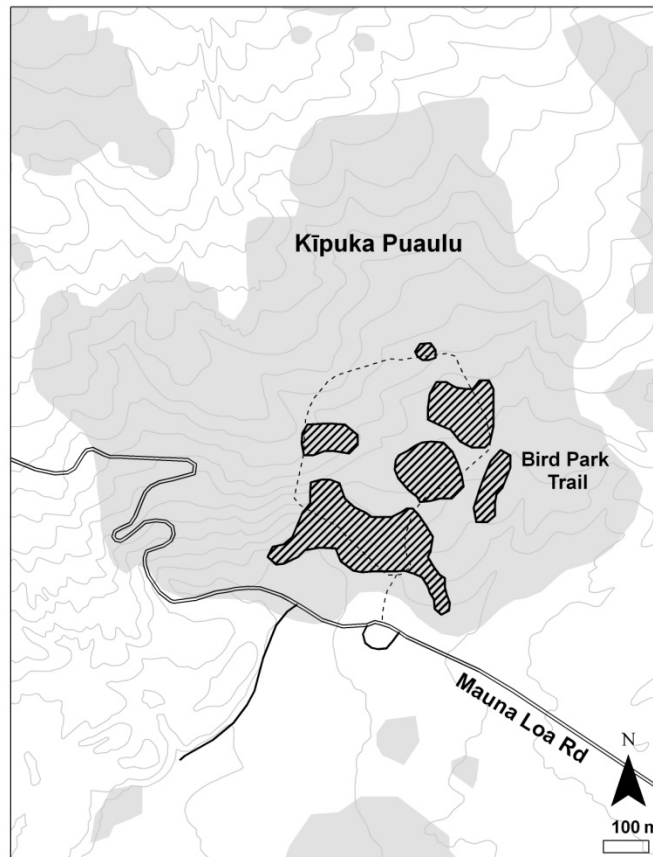


Figure 12. Areas with *Melicope hawaiiensis* trees in Kīpuka Puaulu, HAVO, in 2008.

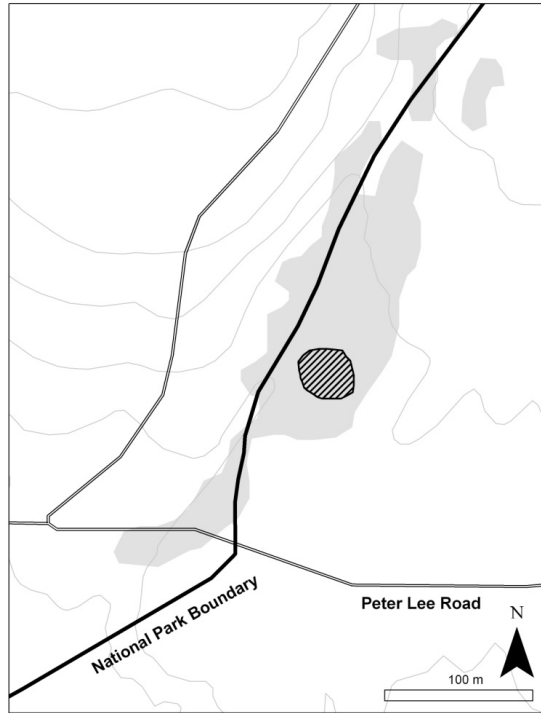


Figure 13. Area with *Melicope hawaiiensis* on the western Park boundary near the Peter Lee Road in 2008.

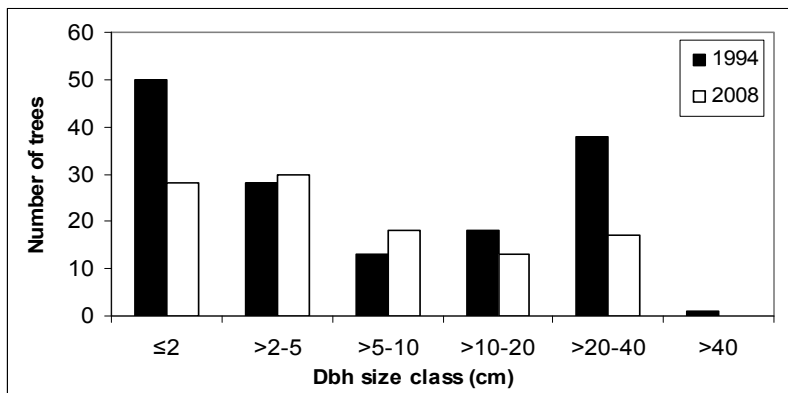


Figure 14. Size class distribution of *Melicope hawaiiensis* trees in Kīpuka Puaulu (and a kīpuka on the western Park boundary), 1994 and 2008.

Growth in diameter of survivors over the 14-year period averaged 1.7 cm (SD = 2.1, n = 114), and the maximum growth detected was 11.1 cm. At the population level there was an overall decrease in height over the 14-year period; the mean decrease was -0.1 m (SD = 1.5, n = 114). The maximum growth in height measured for any *M. hawaiiensis* tree was 5.5 m.

Mortality was pronounced over the 14-year period with 61 trees (38%) dead or not relocated in 2008. There were 12 additional trees found that appear to have been missed during the original survey. Losses were primarily in the large diameter class of >20-40 cm and in the smallest class of <2 cm dbh. These were presumably the oldest and youngest trees of the population. While it is possible that some trees not found in 2008 are still alive, we had specific information on each tree (distance and azimuth from grid points at 25-m intervals), and in many cases, we found either standing dead trunks or logs on the ground at the appropriate location.

Recruitment into the population of *M. hawaiiensis* was detected in 2008. In the course of the study, we found 10 new trees under four cm dbh (and five young trees at the disjunct population of seven on the western Park boundary), and these small-diameter saplings are probably young trees recruited into the population since the survey of 1994. Some of these additions are obviously young plants, such as one first seen in 2005 as a seedling in a grassy meadow that grew quickly to a height above two m. An additional 12 trees found in 2005-2008 were greater than four cm in diameter, and these were almost certainly missed during the original survey and do not represent recruitment in the last two decades.

Reproductive Phenology 2006-2008

The reproductive phenology of 42 randomly selected *M. hawaiiensis* trees in Kīpuka Puauulu and six of seven trees in a kīpuka on the western Park boundary was monitored for more than two years. Buds were seen throughout the year with a higher percentage of trees bearing buds in the winter and spring (Fig. 15). Winter and spring monitoring periods also showed the highest estimated abundance of buds on sampled trees. The proportion of trees with buds was greater than those with flowers on all sampling dates except April 2008 (Fig. 15 and 16). Flowers appeared to be more ephemeral than buds, and flowering peaks were less pronounced. Fewer than 30% of monitored trees bore flowers at any month of 2006 or 2007.

Flowering improved in 2008 when there was a peak in flower production in the spring of 2008 (Fig. 16) There was no clear seasonal pattern of fruit production in *M. hawaiiensis* (Fig. 17 and 18). The differences in fruit production between years were not pronounced, although January 2007 had few trees bearing fruit. In both years fruit production was typically limited to fewer than 20% of monitored trees. Approximately the same proportion of trees bore immature fruit as mature capsules, except in spring months when more trees had immature fruit. Estimated abundance of immature fruit was greatest in summer and fall months, a pattern followed also by mature fruit (Fig. 17 and 18).

Throughout the study, only 48% (n = 48) of monitored trees produced fruit; most randomly-selected trees were not observed bearing either immature or mature capsules over the two-year study. Larger trees were more productive; the mean dbh of trees without fruit was 3.7 cm (SD = 2.6, n = 25), while that of fruit-bearing trees was 13.5 cm (SD = 11.2, n = 23). This difference in diameter of trees with and without fruit was statistically significant ($t = -4.3$, $df = 46$, $p = <0.0001$). There appeared to be no minimum diameter for fruit bearing, because several trees with diameters ranging from 0.6 to 2.6 were observed with immature fruit capsules during the study.

Branch phenology. On three productive trees with reachable reproducing branches we randomly selected seven to nine fertile branches at least one m in length and counted all buds, flowers, immature green fruit, and mature fruit bimonthly for eight months. The mean number of buds per branch was highest in the winter, as was the mean number of flowers (Fig. 19). Tenfold more buds were counted than flowers at monitoring periods. When we were able to reach flowers and distinguish male from female, most flowers were female. Male and female flowers were typically borne on the same inflorescence. The monthly mean number of immature fruit counted per branch averaged between less than one and eight; peak production of immature capsules was observed in spring 2008. Fewer mature capsules were counted on branches; the mean never exceeded two fruit per branch (Fig. 19).

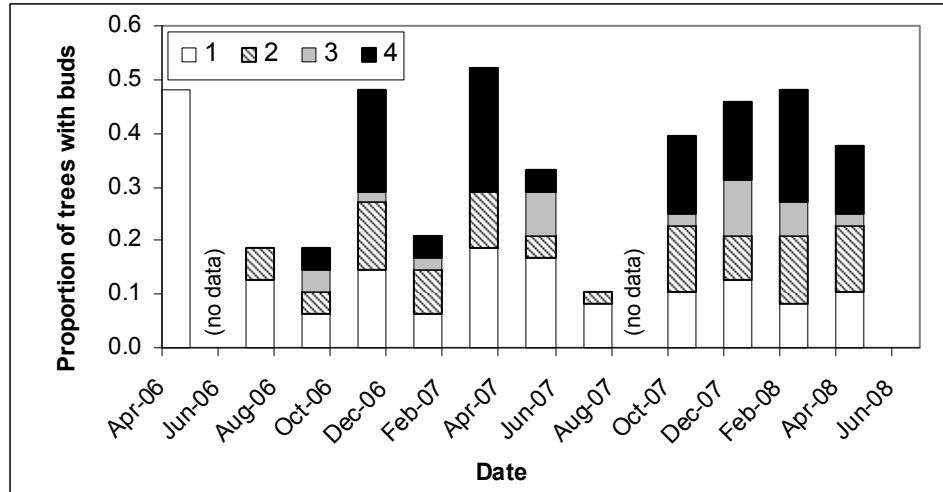


Figure 15. Estimated abundance of buds in four categories for *Melicope hawaiiensis* trees in Kīpuka Puauu and a kīpuka on the western boundary, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

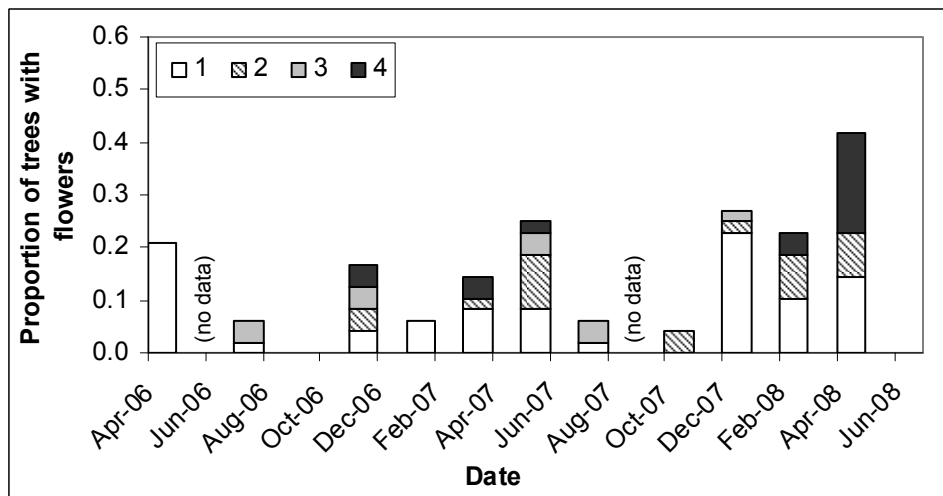


Figure 16. Estimated abundance of flowers in four categories for *Melicope hawaiiensis* trees in Kīpuka Puauu and a kīpuka on the western boundary, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

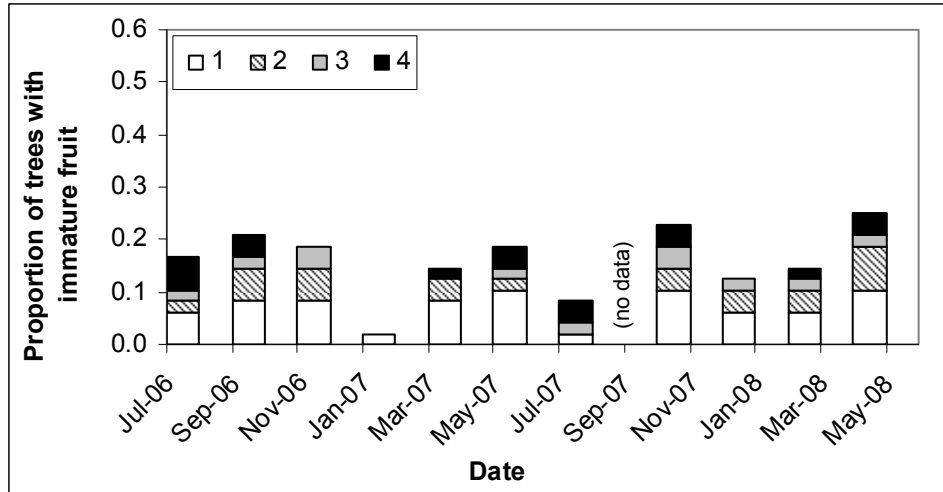


Figure 17. Estimated abundance of immature fruit capsules in four categories for *Melicope hawaiiensis* trees in Kīpuka Puaulu and a kīpuka on the western boundary, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

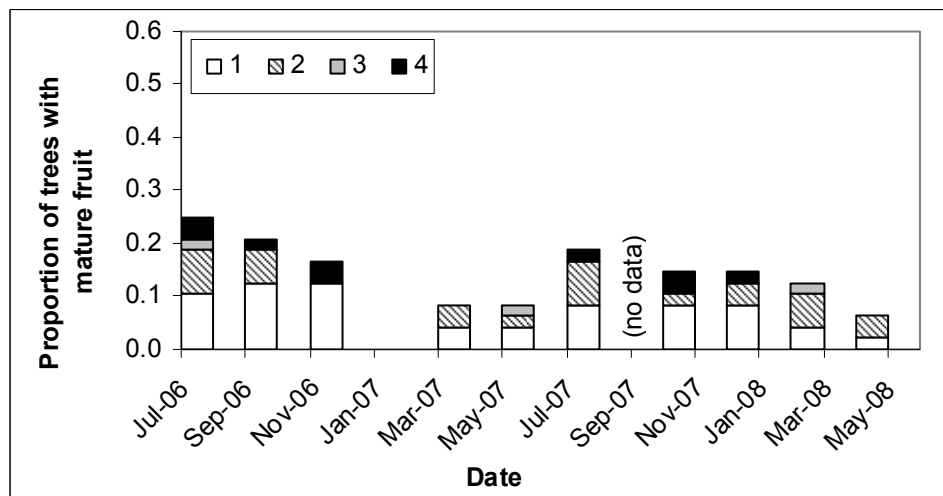


Figure 18. Estimated abundance of mature fruit capsules in four categories for *Melicope hawaiiensis* trees in Kīpuka Puaulu and a kīpuka on the western boundary, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

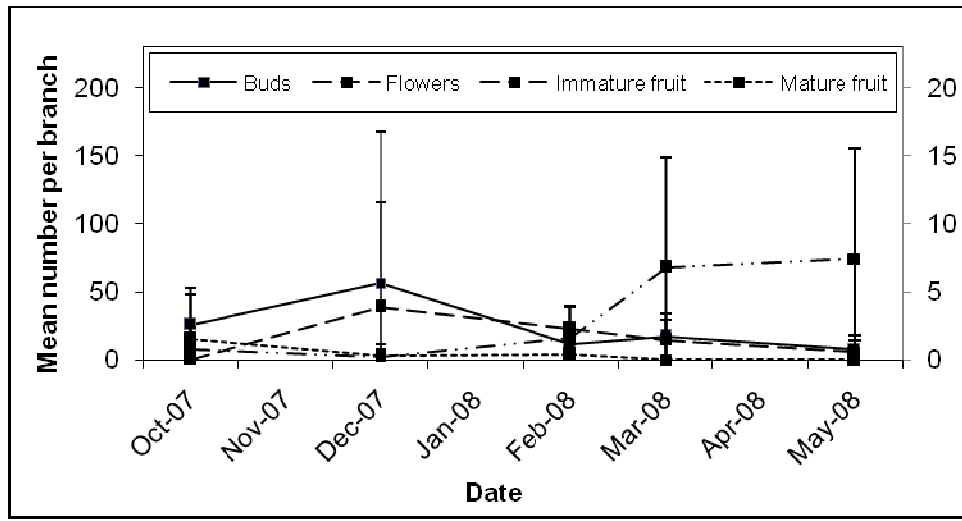


Figure 19. Mean number (\pm SD) of buds, flowers, immature fruit, and mature fruit on sampled branches of three *M. hawaiiensis* trees in Kīpuka Puauulu and a kīpuka on the western boundary, 2007-2008. The left y-axis shows number of buds, and the right y-axis is the number of flowers and fruit.

Success of Fruit Development

Tagged inflorescences containing buds and female flowers on reachable branches of flowering trees were monitored to determine the successful production of immature or mature capsules. Overall, the transition rate of buds and female flowers to immature green fruit was 18% and to mature fruit was 4% ($n = 1023$). When small immature or green fruit were tagged as such, the rate of successful transition to mature fruit was 25% ($n = 513$). On average a tagged bud or flower required 2.0 months ($SD = 2.2$, $n = 108$) to become a green, immature fruit. Tagged immature fruit became mature fruit after an average of 4.5 months ($SD = 1.5$, $n = 87$).

Floral Visitation Rates of Potential Pollinators

The only documented visitor to flowers of *M. hawaiiensis* during the timed observations was *Allograpata exotica* (Syrphidae), an introduced syrphid fly. The endemic koa butterfly *Udara blackburni* (Lycaenidae) and the sap beetle *Prosopius subaeneus* (Nitidulidae) were also observed visiting flowers, but visits were seldom, only occurred in Kīpuka Puauulu, and were

never noted during the timed observations. A total sample of 767 flowers was monitored in 41 observation periods, each of 20-minutes duration. Of the flowers, 535 were male and 232 were female. *A. exotica* visited 46 flowers in total, 37 male and 9 female. Overall, mean visitation rate for *A. exotica* was low (0.17 visits/flower/hr, SD = 0.32, n = 41) (Table 3) with a significant difference between visitation rates to male (0.21 visits/flower/hr, SD = 0.38, n = 32) and female (0.10 visits/flower/hr, SD = 0.27, n = 28) flowers (Mann-Whitney U = 337.0, $n_1 = 32$, $n_2 = 28$, $p = 0.04$). A single floral visit ranged from one second to seven minutes with an average of 38.4 seconds (SD = 86.2, n = 46). No significant difference was found between the average time spent at male (45.2 sec, SD = 95, n = 37) and female (10.6 sec, SD = 12.0, n = 9) flowers (Mann-Whitney U = 156.5, $n_1 = 37$, $n_2 = 9$, $p = 0.78$). *A. exotica* foraged by straddling the corolla lobes and probing the corolla tube with its proboscis for either nectar from the eight-lobed nectaries or pollen from the anthers.

Seed Predation

Seed predation by insects. When insect exit holes were observed on *M. hawaiiensis* fruit capsules, we attempted to quantify the level of insect damage by repeatedly collecting mature or nearly mature fruit on at least three trees and examining them for holes, frass, larvae, pupae, and seeds. The insect responsible for predation is a moth in the family Yponomeutidae, identified to the genus *Prays* (Zimmerman 1978). We do not have specific identification of the insect or insects, but moths collected from both *M. hawaiiensis* and *M. zahlbruckneri* have been tentatively identified as two or more undescribed endemic species of *Prays* (Jon Giffin, pers. comm.) Fruit collections were made in March, July, and September 2008. Overall, 22% of collected capsules showed some sign of insect predation (n = 207) (Table 4). In this total group of capsules, 11% had an insect exit hole, 20% had frass where a consumed seed had been, 10% enclosed a larva or pupa, and 73% contained intact seeds. The single sample tree within Kīpuka Puauulu (MH89) displayed a higher rate of insect predation and a lower number of intact seeds per capsule than did the two regularly-sampled trees at the western boundary kīpuka. Insect predation rates were similar on capsules collected in March and July 2008, but were lower in September (Table 5). The mean number of intact seeds per capsule was similar in both March and September, and fewer intact seeds were noted in July. Overall, the number of intact seeds per capsule was not significantly different in predated and non-predated capsules ($t = 0.4$, $df = 204$, $p = 0.7$).

Seed predation by rodents. Seeds of *M.hawaiiensis* were offered in October 2008, and after two months there was no removal or predation in any of the open stations or controls.

Table 3. Abundance, visitation rates, and duration of time spent by the non-native syrphid fly *Allograpata exotica* at flowers of *Melicope hawaiiensis* in Kīpuka Puaulu and a kīpuka on the western boundary near Peter Lee Road in September 2007 and March 2008.

Sex of Flower	Number of visits	Mean visitation rate ¹ (SD)	Mean visit duration ² (SD)
Male	37	0.21 (0.38)	45.2 (95.0)
Female	9	0.10 (0.27)	10.6 (12.0)
Total: sexes combined	46	0.17 (0.32)	38.4 (86.2)

¹ Rates represent the mean number of flowers visited per open flower per hour for each observation period. Only visits in which the visitor probed the corolla with its proboscis to utilize floral resources were included. Mean visitation rate is based on the average across all observation periods.

² Mean time spent visiting a flower is based on individual visits measured in seconds independent of observation period.

Table 4. Insect predation on collected mature capsules of sampled *Melicope hawaiiensis* trees in Kīpuka Puaulu and a kīpuka on the western boundary, March-September 2008.

Tree code#	# of capsules collected	% predated	Mean # intact seeds/capsule	SD	Mean # aborted seeds/capsule	SD
MH89	70	31	1.3	±1.4	1.5	±1.0
MH158	63	18	3.0	±2.1	2.1	±2.0
MH159	69	17	3.0	±2.2	2.8	±1.5
MH160	5	20	0.4	±0.9	5.6	±2.3
Total	207	22	2.3	±2.1		

Table 5. Monthly insect predation on collected mature capsules of *Melicope hawaiiensis* trees in Kīpuka Puaulu and a kīpuka on the western boundary, March-September 2008.

Month	# of capsules collected	% predated	Mean # intact seeds/capsule	SD	Tree# of sample
March	89	26	3.5	±1.7	89,158,159
July	72	28	0.6	±1.0	89,158,159, 160
September	46	7	2.9	±2.0	89, 158, 159

Observations of rodent predation on capsules. While counting and monitoring tagged inflorescences in summer 2008, we noted rodent predation on many tagged fruit capsules of two of the three intensively sampled *M. hawaiiensis* trees. Predation took the form of chewing into one or more carpels of the capsule and removing the seeds. Some capsules had marks of biting or chewing, but the capsule was not opened. On other tagged fruit, the peduncle attaching the capsule to the branch was severed at an angle, indicating that rodents had cut the attachment with their sharp teeth; in these cases the fruit was missing but the numbered tag remained. In August 2008, the incidence of predation on tagged fruit was 53.8% (n = 78). Predation continued into September, when 57.1% of capsules or stalks of former capsules showed signs of rat predation (n = 14).

Seeds and Seed Germination

Soil seed bank. Soil cores were collected at three sampling periods: June 2008, September 2008, and March 2009. At each of three sampled trees, ten cores were taken and pooled for analysis. In the first sampling, all three trees had both whole and partial *M. hawaiiensis* seeds within the soil seed bank. Fifty-five whole seeds and 1,318 partial seeds were collected from cores; the mean number per tree was 18.3 whole seeds (SD = 9.1, n = 3) and 439.3 partial seeds (SD = 441.6, n = 3). Ninety-six percent of recovered seeds appeared to be predated by rodents and had irregular, gnawed edges. In the second sampling, two of three trees had whole seeds in cores and all had partial seeds. The total number of whole seeds collected was 37 and there were 3,275 partial seeds. Means of whole and partial seeds per tree were 12.3 (SD = 13.7, n = 3) and 1,091.7 (SD = 1,370.9, n = 3), respectively. Almost 99% of material collected was composed of fragments that showed signs of rodent predation.

Whole seeds from the soil were planted in the greenhouse and within two months, seedlings began to appear from the seed bank of one tree sampled in both June and September and a second tree sampled in June. Nine months after planting, germination rates in the greenhouse were 28.6% and 44% for seeds of the first tree and 4.5% for those of the second.

In the March 2009 samples, 79 whole seeds, 146 half seeds split at the suture, and 1,965 predated fragments of seeds were recovered from the soil cores at three trees combined. Most seeds and fragments came from cores beneath the two sampled trees in the kīpuka on the western Park boundary. The mean per tree was 26.3 whole seeds (SD = 16.9, n = 3), 48.7 half seeds (SD = 27.1, n = 3), and 655 predated partial seeds (SD = 609.6, n = 3). Most of the partial seeds were obviously predated with irregular broken fragments and gnawed edges (89.7% of all partial seeds recovered). Whole seeds recovered from the March soil seed bank cores were planted in greenhouse flats. After four months, 10.5% and 8.8% of seeds had germinated from the soil seed bank of two trees; seeds from the third tree showed no signs of germination.

Seed germination in the greenhouse. Three germination trials were carried out on fresh *Melicope hawaiiensis* seeds in the HAVO greenhouse: the first trial began in September 2006; the second in October 2007; and the last was started in August 2008. Germination trials used from ten to 72 seeds in replicated flats. Exact dates of first germination were not always recorded in 2006, but in 2007 the first sign of germination occurred six months after sowing, and in 2008 the first seedlings appeared after two months (Appendix II, Table 2). Germination rates were low in our trials; the mean germination rate in 2006 was 11.1%. In 2007 the mean germination rate was 7.1%. After a year, only one seed germinated (1.4%) in one flat of the 2008 trial; this last trial has not been underway long enough to determine the rate of germination. The first two trials were monitored for 15 months before termination.

Seed viability test. Twenty mature seeds of *M. hawaiiensis* were collected in 2008 and tested for viability 24 hours later. None of the seeds floated during the pre-test imbibing. The mean seed coat thickness was 0.2 mm (SD = 0.1, n = 20). Upon opening of the seed coat, all seeds had healthy-looking endosperm and embryos. The internal parts of the seed were not removed from the bisected halves of the seed coat before treatment. One seed had strongly positive results, twelve had positive results, and six had weakly positive results.

***Melicope zahlbruckneri*, Alani**

Size Class Structure, Growth, and Mortality

Kīpuka Puaulu was systematically searched in 1993-94, and 35 trees of *M. zahlbruckneri* were mapped (Pratt and Abbott unpublished data). The sites of all *M. zahlbruckneri* trees were visited in 2006-2008 (Fig. 20), when 19 trees were found alive and 16 were dead or could not be found. The size-class structure exhibited in 1993 included small and medium-size trees, as well as large trees; the presence of small trees in two size classes <5 cm indicated that some natural reproduction had occurred in the past (Fig. 21). By 2008, three large trees (>20 cm dbh) remained, and most plants of the extant population were in the diameter classes of >2-5 cm and >5-10 cm. There was only one plant found in the smallest diameter class of <2 cm; this is presumably a young plant, although it is more than 15 years old. Average growth in diameter between 1993 and 2008 was 1.9 cm (SD = 1.6, n = 19). Height increased on average 0.8 m (SD = 1.2, n = 19) during the 15-year interval.

Mortality over the interval between surveys was pronounced; 46% of the population alive in 1993 had died by 2008. Mortality was greatest in the larger two diameter classes, where 11 of 14 trees died by 2008. More than half of the trees in the >5-10 cm class died between 1993 and 2008, but several smaller trees grew into this group, resulting in a net increase for the class in 2008. Losses in the two lowest diameter classes were small; only one tree died in each of the 0-2 cm and >2-5 cm groups between 1993 and 2008. The bulk of the small plants alive in 1993 grew into the next highest diameter class by 2008 (Table 6). There was no seedling recruitment detected since the first measurement of the population. The only plant in the smallest size class in 2008 had been observed in 1993 and remained a small plant only slightly taller than a meter.

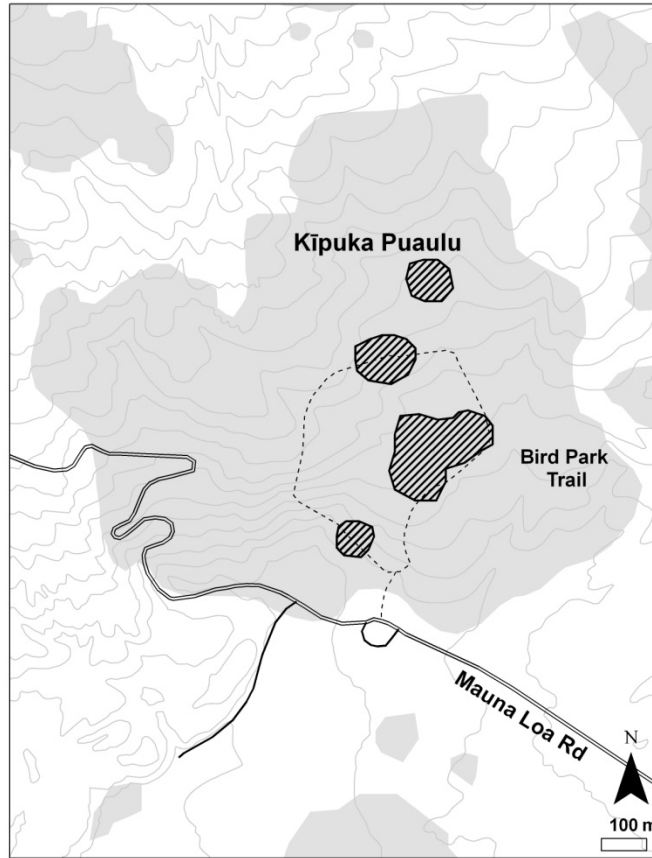


Figure 20. Areas with *Melicope zahlbruckneri* trees in Kipuka Puauulu, HAVO, in 2008.

Table 6. Size class distribution, mortality, and growth into subsequent size classes for *Melicope zahlbruckneri* trees in Kipuka Puauulu between 1993 and 2008.

Size class (cm)	# of trees 1993	# of trees 2008	Mortality (# of trees)	+1 size class	+2 size classes
≤2	8	1	1	5	1
>2-5	9	8	1	5	0
>5-10	5	8	3	0	0
>10-20	8	0	5	3	0
>20-40	7	4	6	0	0
>40	0	0	-	-	-

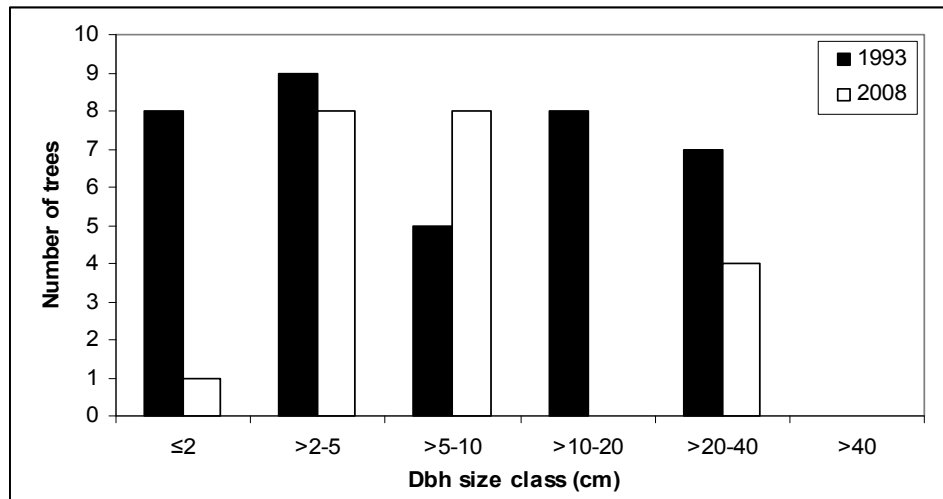


Figure 21. Size class structure of *Melicope zahlbruckneri* in Kipuka Puau, 1993 and 2008.

Reproductive Phenology 2006-2008

Most *M. zahlbruckneri* trees in Kipuka Puau supported reproductive structures at some point during the 2.5-year study period. Overall, buds were produced throughout the year although no clear seasonal pattern existed; only late spring months showed a slight decline in the number of trees bearing buds (Fig. 22). Fewer trees bore flowers; these structures appeared to be more ephemeral than buds and may have been missed during the two-month monitoring interval of 2006 and early 2007 (Fig. 23). There appeared to be a weak peak in flowering in the fall and winter. Most trees had low estimated numbers of inflorescences with buds (<50) throughout the study period, but a few trees had large numbers of inflorescences bearing buds (>100), particularly in 2008. Flowers were less abundant; typically only a quarter or a third of trees had flowers at any monitoring period. No tree was ever estimated to have more than 50 clusters of buds throughout the study, and usually 1-10 inflorescences were noted at those trees bearing flowers.

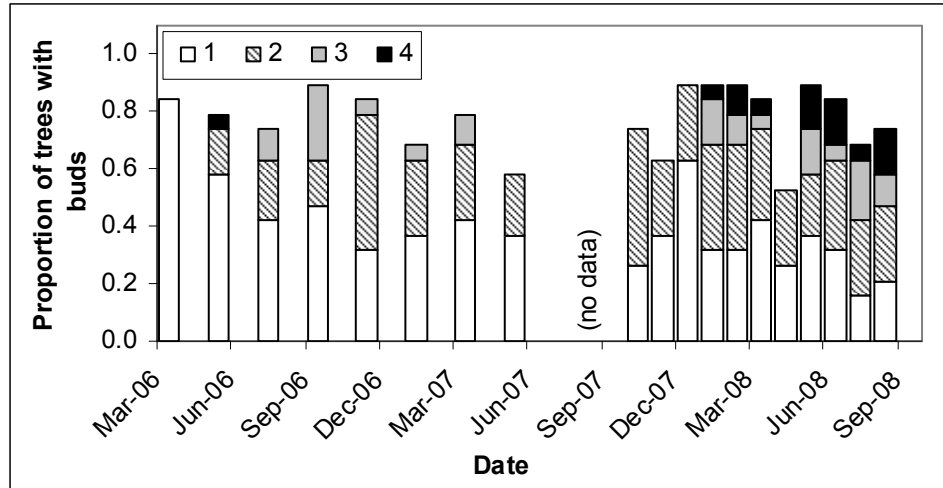


Figure 22. Estimated abundance of buds in four categories for *Melicope zahlbruckneri* trees in Kipuka Puau, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

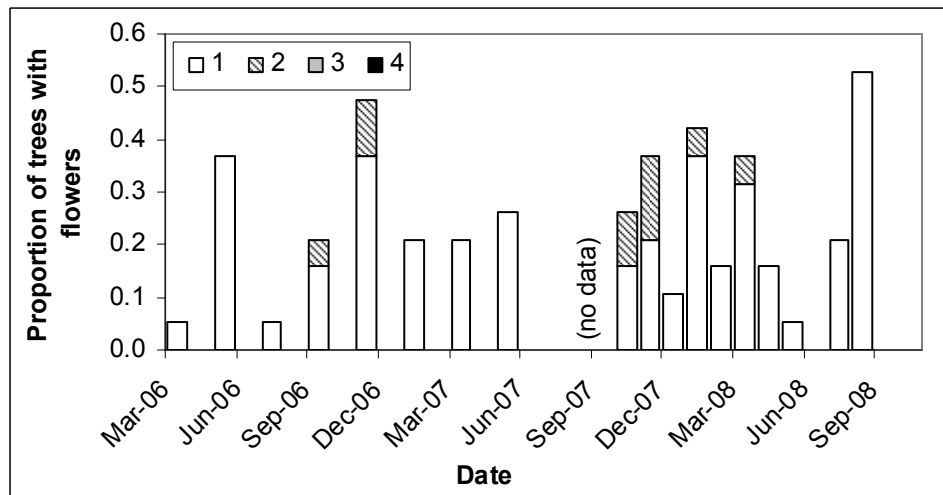


Figure 23. Estimated abundance of flowers in four categories for *Melicope zahlbruckneri* trees in Kipuka Puau, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

Fruit capsules were rarely seen throughout the study, except at one highly productive tree growing in a clearing in the northeastern quarter of the kīpuka, and only five trees were observed with mature fruit during the entire study. Less than 10% of *M. zahlbruckneri* trees bore fruit (either immature or mature) during 2006 and 2007, except in November 2007 when 15% of trees had immature fruit (Fig. 24). Fruit production improved during the monitoring year 2008, when approximately one third of trees had immature fruit in the winter, and 15% were observed with mature fruit in March and May. Immature fruit required four months on average to mature from the time they were first noticed, although they could persist as immature, green fruit for as long as eight months. Fruit contained mature seeds when they were mottled green, but had enlarged to 19 mm in width (the mean width of mature, mottled to brown capsules). The monthly mean number of mature fruit counted on all trees of the population never exceeded two, although the means of immature fruit counted were slightly higher (Fig. 25).

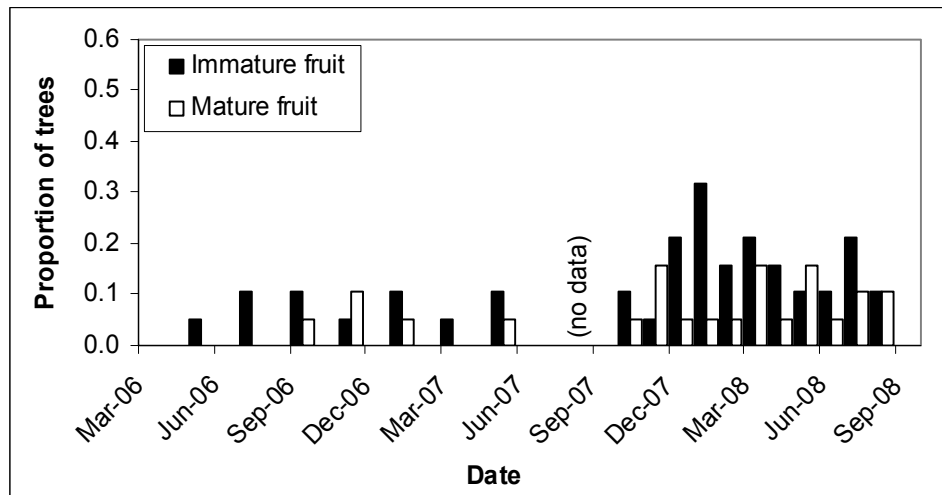


Figure 24. Phenology of fruit production in *Melicope zahlbruckneri* trees in Kipuka Paaulu, 2006-2008.

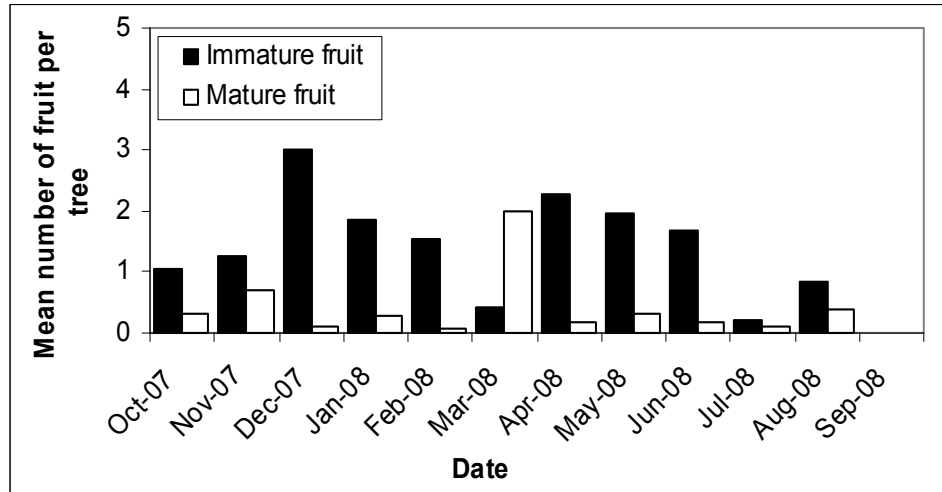


Figure 25. Mean number of fruit capsules on *Melicope zahlbruckneri* trees in Kipuka Puauu, October 2007 to September 2008.

Success of Fruit Development

Tagged inflorescences containing buds and flowers on reachable branches of flowering trees were monitored to determine the transition rate to immature, green capsules. On several trees that produced fruit, immature green capsules were also tagged and monitored to follow the successful transition to mature capsules and to determine the time required for maturation of fruit. The transition rate of tagged inflorescences with buds and female flowers to immature, green fruit was very small, amounting to only 0.5% throughout the study ($n = 1123$). The transition of buds and flowers to mature fruit was even smaller at 0.2% ($n = 1123$). The actual rate of transition reported here likely includes some buds that became male flowers and could not have become fruit. Male and female flowers appear to be borne on the same inflorescences, and flower gender cannot be determined in the bud stage. Flowers were ephemeral, but buds were persistent for several months. The average development time of a bud to an immature fruit was 3.0 months ($SD = 2.0$, $n = 4$).

Even though the successful transition rate of bud or flower to immature or mature fruit was very low, the chance of successful production of a mature fruit from an immature one was much higher. Forty percent of tagged immature, green fruit became mature capsules, although not all of these produced seeds (see Seed predation by insects section below). Based on

tagged capsules, the mean time required for an immature fruit to develop into a mature capsule that was mottled green or brown in color was 4.0 months (SD = 1.6, n = 4).

Understory Thinning Experiment

Vegetation cover at treatment and control trees. There were five understory thinning plots from which *Coprosma rhynchocarpa* was removed and five control trees, but the thinned plots had a total of nine *M. zahlbruckneri* trees within them because five trees were clustered together at one site. Prior to treatment, *C. rhynchocarpa* was the species with the highest amount of cover in both plot types, comprising 25-50% in low (1-3 m) and middle (>3-10 m) layers (Table 7). *Psychotria hawaiiensis* var. *hillebrandii*, *Pisonia brunoniana*, *Pipturus albidus*, and *Sapindus saponaria* were also prominent in the understory vegetation surrounding *M. zahlbruckneri*.

Effects of understory thinning. In the nine-month period following removal of the understory dominant *C. rhynchocarpa* from treatment trees, we detected no positive effect of understory thinning on the reproduction of *M. zahlbruckneri*. Trees in understory-removal plots were more often sterile than were control trees, and there was a higher estimated occurrence of buds on control trees ($\chi^2 = 10.5$, df = 4, p = 0.033). There was no statistically significant difference in the distribution of flowers ($\chi^2 = 8.9$, df = 4, p = 0.06) or average number of immature fruit capsules (t = 1.8, df = 14, p = 0.97) between treatment and control plots. We observed 17 immature fruit capsules on control trees and 10 young fruit on trees in treated plots. Over the nine-month observation period there was a total of 12 mature fruit observed on two control trees and no mature fruit on treatment trees, but the difference was not significant (t = 1.1, df = 6, p = 1.94). We considered fruit to be new on a tree in a given month, if the previous month had no fruit detected.

Table 7. Estimated cover-abundance of all woody species in three height layers at selected *Melicope zahlbruckneri* trees in Kīpuka Puʻaʻulu.

Control trees (n=5)				
Species Present	# of trees where species occur	% cover 1-3m	% cover >3-10m	% cover >10m
<i>Acacia koa</i>	1	0	0	50-75
<i>Coprosma rhynchocarpa</i>	5	25-50	25-50	<1
<i>Ipomoea indica</i>	5	1-5	1-5	0
<i>Metrosideros polymorpha</i>	5	0	0	1-5
<i>Myrsine lessertiana</i>	2	0	<1	<1
<i>Pipturus albidus</i>	4	<1	1-5	<1
<i>Pisonia brunoniana</i>	3	1-5	1-5	0
<i>Psychotria hawaiiensis</i> var. <i>hillebrandii</i>	5	5-25	5-25	0
<i>Sapindus saponaria</i>	5	1-5	1-5	1-5
<i>Sophora chrysophylla</i>	4	<1	<1	<1

<i>Coprosma</i> removal trees (n=9)				
Species Present	# of trees where species occur	% cover 1-3m	% cover >3-10m	% cover >10m
<i>Acacia koa</i>	0	0	0	0
<i>Coprosma rhynchocarpa</i>	9	25-50	25-50	<1
<i>Ipomoea indica</i>	9	1-5	1-5	<1
<i>Metrosideros polymorpha</i>	9	0	0	5-25
<i>Myrsine lessertiana</i>	8	0	0	1-5
<i>Pipturus albidus</i>	3	0	1-5	<1
<i>Pisonia brunoniana</i>	4	1-5	1-5	0
<i>Psychotria hawaiiensis</i> var. <i>hillebrandii</i>	9	5-25	<1	0
<i>Sapindus saponaria</i>	4	0	0	5-25
<i>Sophora chrysophylla</i>	4	<1	<1	<1

Seed Predation

Seed predation by insects. In total, 124 fruit were collected, primarily from one large and productive tree, but a few capsules were found on three other trees. For the monthly samples of collected fruit, insect predation evidenced by exit holes, frass, larvae, pupae, or predated seeds ranged from 67 to 100% (Table 8). Incidence of insect predation was high at all sampling periods, and few intact seeds remained in predated capsules. The highest number of intact seeds per capsule was observed in late summer; this was also the period showing the

lowest incidence of insect predation. For the most productive tree, the rate of fruit predation was 82% (n = 114), and an average of only 1.1 (SD = 1.7) intact seeds per capsule were collected during the monthly sampling. The mean number of carpels developed on 67 collected capsules was 3.2 (SD = 0.9).

Table 8. Insect predation on collected mature capsules of *Melicope zahlbruckneri* trees in Kīpuka Puʻaʻulu 2007-2008.

Month	# of capsules collected	Tree code#	% predated	Mean # intact seeds/capsule	SD
Dec	32	36	75	0.3	±1.0
Jan	19	3,28,36	95	1.0	±1.5
Feb	29	36	93	0.2	±0.5
Mar	5	3,36	72	0.8	±0.5
Jun	18	3,22,36	72	2.6	±1.8
Jul	3	36	100	1.7	±2.0
Aug	12	3,36	75	3.1	±2.4
Sep	6	36	67	2.5	±1.0

Seeds and Seed Germination

Soil seed bank. The seed bank beneath three fruiting *M. zahlbruckneri* trees was examined in October 2008 and February 2009. In 2008 no intact seeds were recovered from the soil cores, but at two trees we found partial seeds in the pooled cores. One productive tree had seven partial seeds in the core samples, and another had one partial seed. The seeds appeared to have been predated by rodents; only the empty seed coats remained and these were irregularly gnawed. In winter 2009, only one non-predated partial seed was found in the soil at one tree.

Seed germination in the greenhouse. Few seeds were available on *M. zahlbruckneri* trees until fall 2007, when a few mature capsules were collected, primarily at one tree. One replicated seed germination trial was carried out at the HAVO greenhouse in October 2007 with three freshly-collected seeds in each of five pots. No germination was observed until March 2009, when one seedling appeared 1.5 years after planting (6.7%). A germination trial using 43 seeds in each of three replicates was underway at the end of the study; no germination was

seen in seven months. Later collections of mature *M. zahlbruckneri* seeds in June and August 2008 were transferred to HAVO Propagation Specialists.

In 2008, three mature capsules containing ten seeds were sent to Nellie Sugii at the Lyon Arboretum in Honolulu. She treated the outside of the capsules with disinfectant, removed seeds, and propagated them using tissue culture techniques at her laboratory. One of these seeds germinated after 44 days.

The two seedlings from these trials are the only successful germination attempt with this species recently, other than one seedling from an earlier propagation attempt by HAVO Rare Plant Specialists; the earlier seedling and three successfully rooted cuttings were planted in Kīpuka Kī in 2002, where they have persisted, and by summer 2007, attained an average height of 1.2 m (Thomas Belfield pers. comm.).

Seed viability test. Nineteen *M. zahlbruckneri* seeds were collected from mature green fruit, stored at 5 C° for three months, and tested for viability. No seeds floated; however, two were hollow when bisected. The rest of the seeds appeared healthy on the inside. The mean seed coat thickness was 0.6 mm (SD = 0.2). All had positive results in the viability test.

***Zanthoxylum dipetalum* var. *dipetalum*, Kāwa`u**

Size Class Structure, Growth and Mortality

All known trees of *Z. dipetalum* var. *dipetalum* were mapped in Kīpuka Puaulu in 1993 (Pratt and Abbott unpublished data). When these trees were visited in 2006-2008 and re-measured, 53 live trees were found (Fig. 26). Thirteen trees had died since 1993, and five trees not seen in 1993 were found in the kīpuka; with one exception, new trees were too large to be recruits since the previous mapping.

The size-class structure exhibited in 1993 was an inverse-J curve, indicating a population increasing with the addition of a large number of young (small size class) trees (Fig. 27). This 1993 size class structure is likely explained by the fact that many trees of this species were planted in Kīpuka Puaulu by N. G. Zimmer in the 1970s (Zimmer unpublished). The 2008 size class structure differed with few trees in the smallest size class (<2 cm dbh) and an increase in trees with dbh of 5-10 cm. The trees with current diameters of 5-10 cm apparently moved into this class from below as they grew in diameter over the last 15 years. There was little change

in the largest three diameter categories; large (and presumably old) trees grew little over 15 years. Mean increase in diameter of trees between 1993 and 2008 was 2.4 (SD = 2.6, n = 53) cm, indicating a relatively low growth rate and great variation among trees.

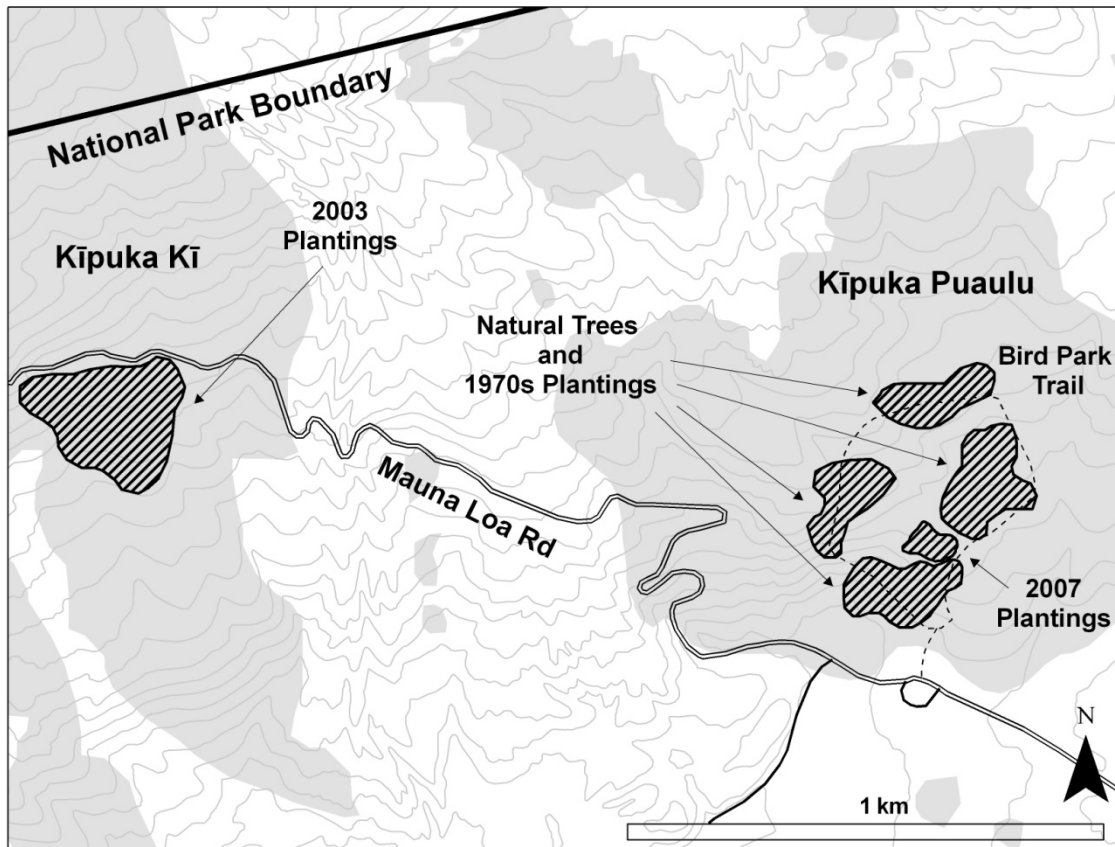


Figure 26. Areas with *Zanthoxylum dipetalum* var. *dipetalum* trees in Kīpuka Puāulu and Kīpuka Kī, HAVO, in 2008.

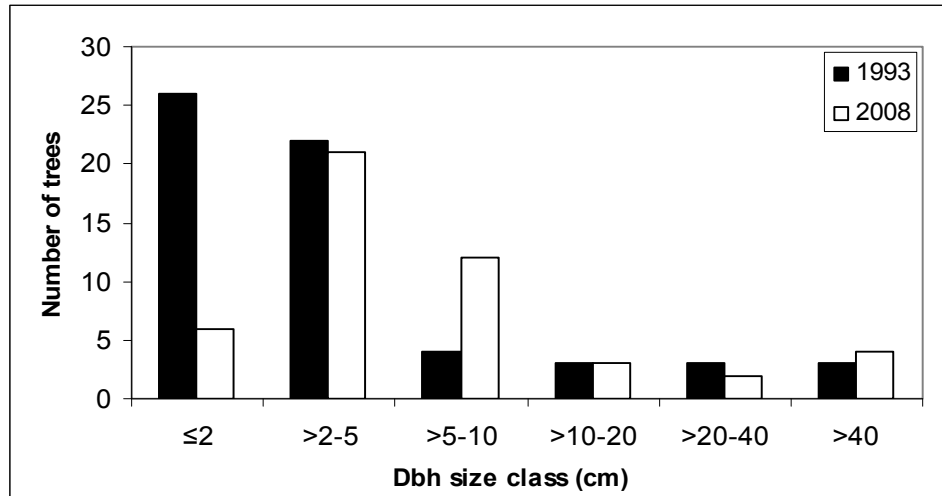


Figure 27. Size class structure of *Zanthoxylum dipetalum* var. *dipetalum* in Kipuka Puau, 1993 and 2008.

Mortality was pronounced over the 15-year period; 20% of trees died between 1993 and 2008. Most of the deaths (seven trees) occurred in the smallest diameter class (<2 cm); these were probably the youngest trees. Two trees were lost from each of the next highest diameter classes (>2-5 and >5-10 cm), and one tree died in each of the higher size classes (>10-20 and >20-40 cm). None of the largest trees died during this time interval.

The gender of most of these dioecious trees was determined during the study (Fig. 28). Male trees (seven) were better represented in the largest three diameter classes than were female trees (four). Numbers of males and females were roughly equal in the middle size class (>5-10 cm); where gender could be determined in the smallest diameter classes (<5 cm), females predominated. Most trees 2-5 cm in diameter (dbh) failed to produce flowers throughout the study, and their gender remains unknown. No trees <2 cm in diameter flowered during the study.

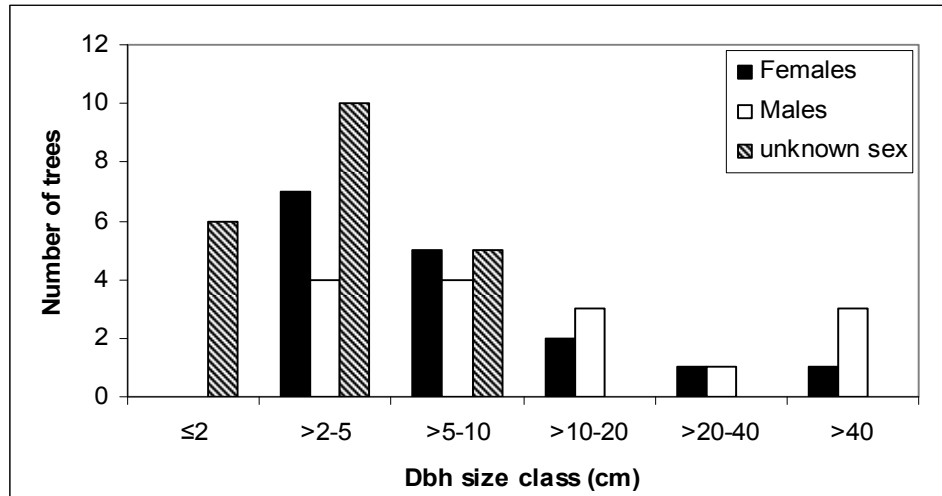


Figure 28. Gender and size classes of *Zanthoxylum dipetalum* var. *dipetalum* trees in Kīpuka Puaulu, 2008.

Reproductive Phenology 2006-2008

More than half of the *Z. dipetalum* trees in Kīpuka Puaulu flowered during the study period; these were typically the trees > 5 cm in diameter. Buds and flowers were seen throughout the year with most trees producing buds during winter and spring months. Fewer trees displayed flowers, and the monthly pattern was less pronounced than for buds. Approximately a third of trees bore flowers during fall and spring months, and a slightly higher proportion had flowers in the winter. Summer months showed less production of both buds and flowers (Fig. 29 and 30). Typically, the estimated abundance of buds was low, in the 1-10 or 11-50 inflorescence categories. Fewer trees bore more than 100 inflorescences with buds, and these displays were seen in winter and spring. Very few trees had flowers with an estimated abundance above 50 inflorescences; most trees with flowers were estimated to have 1-10 inflorescences.

Fruit production was not consistent among years. More than a quarter of female trees bore green, immature fruit during winter and spring months, and a lesser proportion of trees had fruit at other times of the year (Fig. 31). Green, immature fruit were more often seen than were mature fruit; green fruit may persist for more than eight months before they fully ripen to brown and open to reveal seeds. Less than 20% of female trees had brown, mature fruit at

any monitoring period; summer months were a low point for mature fruit (Fig. 32). Once fruit turned brown, they dispersed seeds quickly, became dry, and fell off the trees. High categories of immature fruit were noted on many female trees during peak production months. Typically more than half of trees that bore immature fruit were estimated to have more than 50 infructescences with these green fruit. By contrast, mature fruit were usually estimated as less than 50 infructescences, and most trees were estimated to have less than 10 of these clusters of brown, often dehisced fruit (Fig. 32).

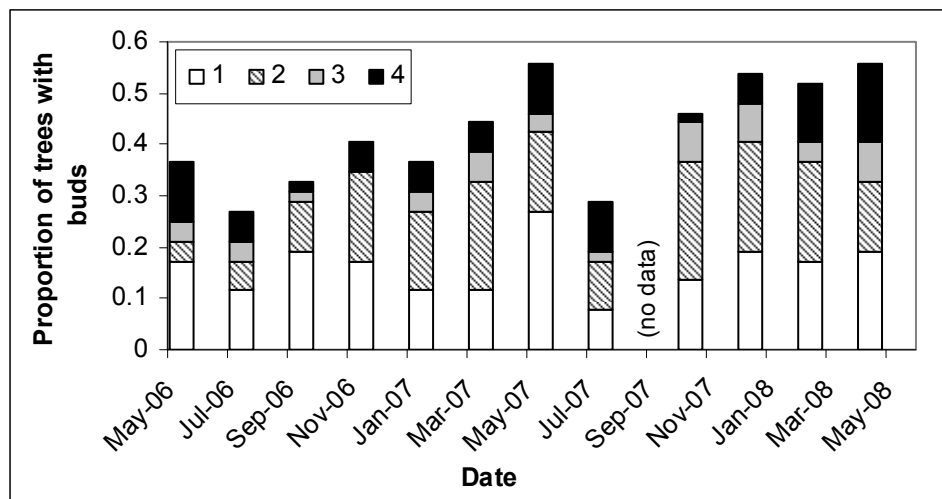


Figure 29. Estimated abundance of buds in four categories for *Zanthoxylum dipetalum* var. *dipetalum* trees in Kipuka Puau, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

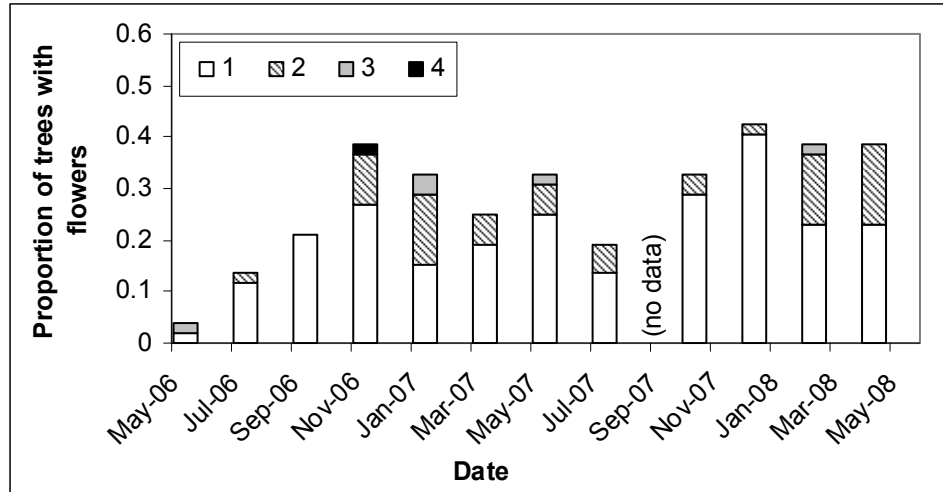


Figure 30. Estimated abundance of flowers in four categories for *Zanthoxylum dipetalum* var. *dipetalum* trees in Kīpuka Puau, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

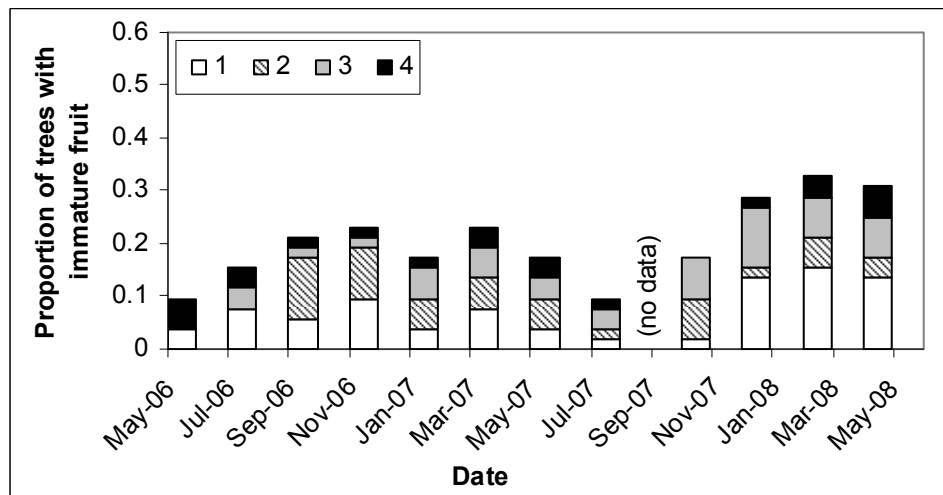


Figure 31. Estimated abundance of immature fruit in four categories for female *Zanthoxylum dipetalum* var. *dipetalum* trees in Kīpuka Puau, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

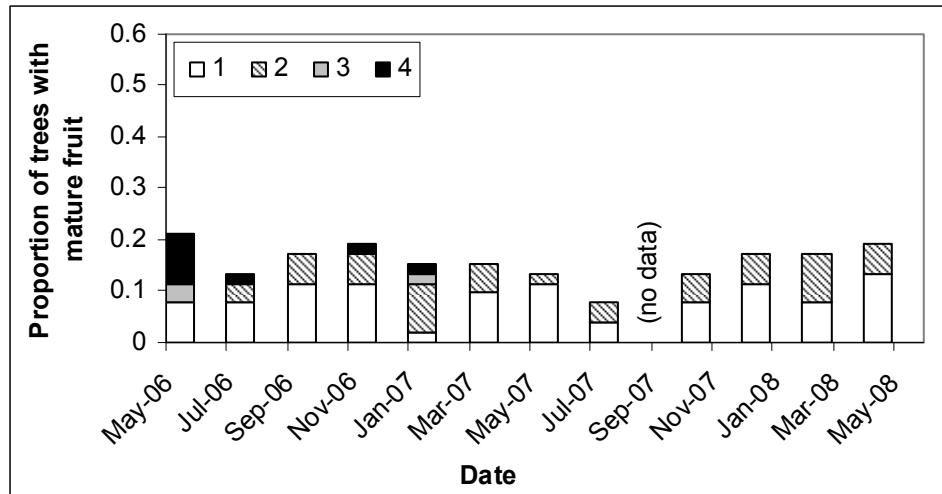


Figure 32. Estimated abundance of mature fruit in four categories for female *Zanthoxylum dipetalum* var. *dipetalum* trees in Kipuka Puauulu, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

Success of Fruit Development

Tagged inflorescences containing buds and flowers on reachable branches of female trees were monitored to determine the transition rate to immature, green fruit. A few small green fruit were also monitored to follow the successful transition to large green and mature fruit. The study began in spring 2006 when a large crop of buds/flowers was available and continued on a smaller scale into summer 2008. At the beginning of the study, some male trees were included in the monitoring; when these trees were identified to gender, their tagged buds were deleted from the data set. Buds and flowers became small green fruit (with enlarged ovaries and styles after the petals fell) with a success rate of 32% (n = 461). Sixteen percent of tagged buds and flowers successfully transitioned through the small fruit stage to become large green, immature fruit. Combining the small green fruit tagged as such with the small green fruit that developed from previously tagged buds and flowers, the transition rate to large green fruit was 47% (n = 191). The successful transition rate of large green fruit to mature, brown fruit was 28% (n = 170), this is artificially low because the monitoring interval of two months was not short enough to preclude dispersal and loss of mature fruit. On average, large green fruit required 8.0 months (SD = 4.0, n = 32) to ripen into mature fruit.

Rat Exclusion Trees

There was no mortality among the 20 selected young plants during 1.5 years of monitoring, and the mean growth since planting in 2002 was 67.9 cm (SD = 11.0). The mean height of selected seedlings was 10.0 cm (SD = 1.6, n =20) when they were planted, and by 2008 they had achieved a mean height of 78.0 cm (SD = 50.6, n =20). Exclosure and unprotected plants were similar in height, although caged plants reached a plateau with a lower maximum height, probably because the wire top of the exclosures inhibited sapling growth at the end of the study. No rat damage was observed during the monitoring period, and there was no significant difference between the growth in height ($t = -0.6$, $df = 19$, $p = 0.59$) or number of leaves ($t = -1.2$, $df = 19$, $p = 0.59$) of caged and uncaged plants.

Seed and Seedling Predation

Seedling predation by Kalij pheasants - Seed plots in and outside exclosures. Two field seeding trials were attempted in Kīpuka Puʻaʻulu with fresh *Z. dipetalum* seeds. In the first trial in May 2007, 22 seeds were planted in each of six plots one m² in size; three plots were grouped on the south side of a meadow and three were on the north side in an area near but not adjacent to existing *Z. dipetalum* trees. Both groups of plots were in partial shade beneath native trees in areas with very little alien grass cover. First germination was noted five months after planting, and seedlings continued to germinate for 15 months until August 2008. By the end of the study, a total of 22 seedlings had germinated in the six plots (16.8% of sowed seeds), with an average of 3.8 per plot (SD = 2.0, n = 6).

Because Kalij pheasant predation was noted on seedlings (bite marks on leaves), pheasant-proof poultry-net exclosures were installed on three of the six seed plots in February 2008. Survival of seedlings before installation of exclosures was zero, and after placement of the netting, survival was 38% inside and zero outside (Fig. 33); this survival rate was significantly higher in the protected plots ($\chi^2 = 3.7$, $df = 1$, $p = 0.05$). In November 2008 both surviving seedlings in unprotected plots showed Kalij pheasant bite marks on leaves and were estimated to have poor vigor; by February 2009, all unprotected seedlings had disappeared in this trial.

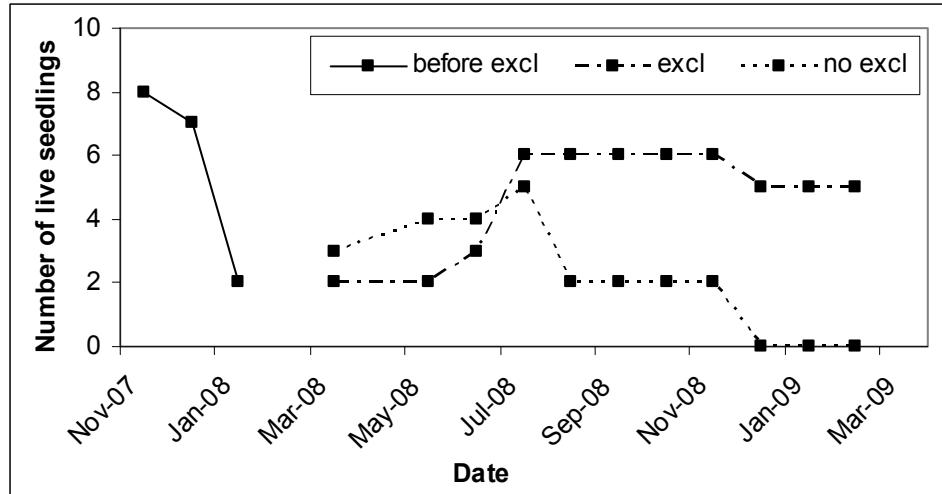


Figure 33. Number of live *Zanthoxylum dipetalum* var. *dipetalum* seedlings in six plots of seeding trial one in Kīpuka Puauulu, 2007 to 2009. (Kalij pheasant exclosures installed in February 2008.)

In the second seeding trial in February 2008, 40 fresh *Z. dipetalum* seeds were planted in each of six plots one m² in size on the south side of the meadow adjacent to three plots of the first trial. Three of the plots (randomly assigned) were protected from Kalij pheasants by poultry netting, and three were unprotected. Germination was first noted three months after planting. After nine months, a total of 21 seedlings had appeared in exclosures (mean 7.0 per plot, SD = 3.6, n = 3), and 28 seedlings were counted in open plots (mean 9.3 per plot, SD = 2.1, n = 3), amounting to germination rates of 17.5 and 23.3%, respectively. The difference in germination rates was not significant ($\chi^2 = 1.3$, df = 1, p = 0.26). After one year there were 14 live seedlings within exclosures and 17 remaining seedlings outside (Fig. 34). Survival was 66.7% inside the exclosures and 60.7% outside; this small difference in the two treatments was not statistically significant ($\chi^2 = 0.18$, df = 1, p = 0.67). In November 2008, 70% of surviving seedlings outside exclosures showed evidence of Kalij pheasant damage in the form of bite marks on leaves or browsed seedling tips.

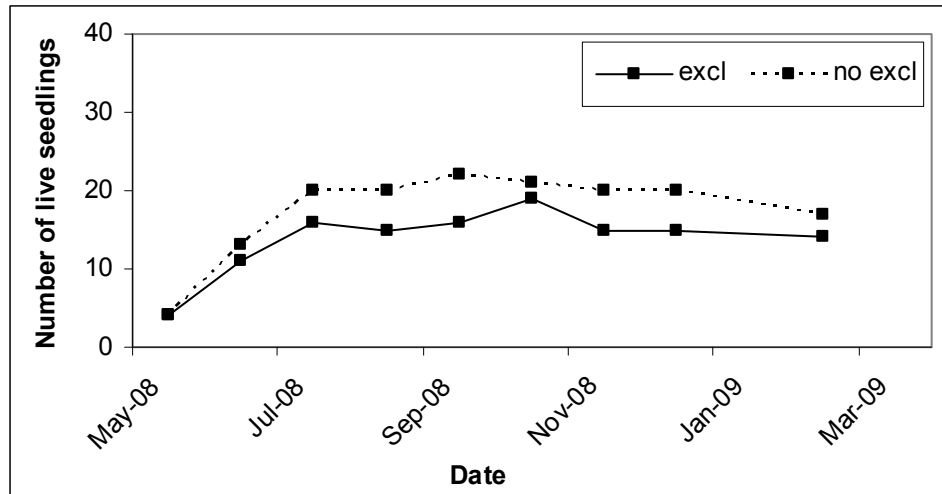


Figure 34. Number of live *Zanthoxylum dipetalum* var. *dipetalum* seedlings in three exclosures and three open plots of seeding trial two in Kipuka Puau, 2008.

Seedling predation by Kalij pheasants - Natural seedlings. In June 2008, natural seedlings were noted beneath several female *Z. dipetalum* trees, and circles of pheasant-proof fencing were installed at five trees, enclosing about one-third of natural seedlings present. Over a nine-month period, a total of 52 seedlings appeared, 16 inside exclosures and 36 outside.

By February 2009 there were 28 surviving seedlings; 75% of exclosure seedlings remained alive, and 44% of seedlings persisted outside. This amounted to a significantly higher survival of seedlings in areas protected from Kalij pheasants ($\chi^2 = 6.5$, $df = 1$, $p = 0.01$, $n = 52$). Mean height of all seedlings was 7.1 cm (SD = 2.0, $n = 28$) in October 2008.

Five seedlings outside exclosures exhibited bite marks on leaves in August, and one was uprooted by digging in September. By November, eight seedlings outside exclosures showed bird bite marks on leaves or were decapitated. Single seedlings had slug slime on leaves in both September and November. In February 2009, bite marks were no longer obvious on leaves, but 29% of seedlings were decapitated and are unlikely to persist. By contrast, 72% of all seedlings, regardless of exclosure type, showed insect damage to leaves or cotyledons in October. A similar percentage of seedlings had insect damage to leaves in February 2009 (68%); 18% had heavy damage with more than half the leaf surface impacted.

Seed predation by rodents. Two offerings of seeds were made during the study to detect rodent predation. In the November 2006 offering, no seeds were predated or lost in either the caged or open treatments over the course of two months. In September 2008, the seed offering was repeated with caged and open offerings adjacent to each other within a circle of large mesh fencing set up to exclude Kalij pheasants from beneath the selected tree. Only one seed was missing from one of the open trays after one month; no other seeds were taken or disturbed in either treatment over a period of three months.

Seedlings with and without Grass

In May 2007, 50 seedlings from germination trials were planted in Kīpuka Puauulu, half at each of two sites: a treeless sunny meadow dominated by alien grasses (primarily *Cynodon dactylon*, Bermuda grass) and an adjacent shady site with leaf litter and no grass cover beneath native tree canopy. When planted, seedlings in the sunny meadow averaged 6.7 cm (SD = 1.2, n = 25) tall, and those in the shade of trees were 4.3 cm (SD = 1.1, n = 25) in height. In July, within two months of planting, all but one of the sun seedlings were dead, and the last survivor disappeared after four months. By contrast, shade-planted seedlings lasted longer; only five had succumbed within two months, and 12 had died by September (four months). The 13 remaining shade seedlings continued to disappear, and by the end of a year only two seedlings were still alive (8% survival). By February 2009, all seedlings were dead or missing. While there was no difference in ultimate survival of seedlings in sun or shade, the mean lifespan of shade seedlings (4.7 months, SD = 4.1, n = 25) was significantly longer than that of sun seedlings (0.7 months, SD = 0.7, n = 25) ($t = 4.9$, $df = 48$, $p = 0.00001$). Overall 24% of seedlings increased in height over the year; the mean growth was 0.8 cm (SD = 0.4, n = 50). More shade seedlings showed growth (36%) than did sun seedlings (12%). Predation and damage to seedlings attributed to Kalij pheasants were noted on 21 seedlings (42%) during the study. Digging for dust baths uprooted several seedlings, and others displayed bite marks on leaves or were defoliated.

Seeds and Seed Germination

Soil seed bank. The seed bank beneath five female trees that were heavily fruiting was examined in December 2007. Four of the five sampled trees had either intact or partial seeds in the soil seed bank, but numbers of seeds recovered were very low. Only two intact seeds

and three partial seeds were found in all the cores sampled. No germination was recorded from the intact seeds planted in the HAVO greenhouse and followed for six months. In a second soil seed bank sampling in December 2008, there were whole *Z. dipetalum* seeds (one and two seeds) in samples at two trees and a partial seed in the soil at one additional tree. No *Z. dipetalum* seeds or remnants were found in soil samples at two of the five trees. The partial seeds showed no sign of predation, but appeared to be split at the suture, perhaps after germination. Seeds and seed capsules of other native tree species were found in cores at all *Z. dipetalum* trees. Low numbers of seeds of forest dominants and common understory species were recovered; species represented were *A. koa*, *C. rhynchocarpa*, *M. polymorpha*, *M. lessertiana*, *S. saponaria*, and *S. chrysophylla*.

Zanthoxylum dipetalum var. *dipetalum* seeds are the largest of the five species studied. The mean length of a sample of seeds collected from multiple trees in 2008 was 18.6 mm (SD = 1.6, n = 25) and the mean width was 14.1 mm (SD = 1.1, n = 25).

Seed germination in the greenhouse. Three *Z. dipetalum* germination trials were carried out in the NPS greenhouse at Hawai`i Volcanoes in 2006, 2007, and 2008. All three trials had three replicate flats and used between 30 and 91 seeds per replicate. The first seedlings appeared within 50 to 64 days after sowing. Germination trials continued for seven to ten months; seedlings continued to germinate from seeds planted 10 months earlier. Mean germination rates for each of the three trials were 56.7%, 56.7%, and 55.3% (Appendix II, Table 3).

Seed viability test. Thirty seeds of *Z. dipetalum* came from three different trees (ZD 32, 51, and 61), where they were collected from the ground or as mature fruit on the tree. Overall, ten seeds floated during imbibing and were hollow when opened (two from ZD 32 and eight from ZD 51). One hollow seed had residue of decomposed endosperm inside, and another showed signs of predation by an insect, probably a moth as indicated by an exit hole and a partial pupal case. In general, the tested seeds appeared healthy.

Eight seeds from ZD 32 were tested: three were strongly positive; three were positive; and one was weakly positive. Of ten seeds from ZD 61, five had strongly positive results and five had positive results. Only two seeds from ZD 51 were tested, one tested strongly positive,

the other positive. Of all *Z. dipetalum* seeds tested, there were nine strongly positive, ten positive, and one weakly positive.

***Sicyos macrophyllus*, `Ānunu**

Sicyos macrophyllus is known in HAVO from only two natural populations: one in lower Kīpuka Kī, discovered in 1996-2000; and a second site near 1,550 m elevation on the western edge of Kīpuka Mauna`iu found by Natural Resources Management Division volunteers in 2005. Because the second group of plants is disjunct from Kīpuka Kī, relatively remote, and difficult to access, it was not monitored in this study. Within Kīpuka Kī, we found four sites with this vine climbing on native trees (Fig. 35); previous population size is unknown, but live plants have persisted at the four sites for more than eight years. Seedlings resulting from germination trials in the current study were planted by HAVO Natural Resources Management staff at both Kīpuka Puaulu and Kīpuka Kī, and we monitored these for a year.

Reproductive Phenology 2006-2008

Because the sprawling life form of *S. macrophyllus* made it impossible to discern individuals, we monitored phenology using 19 randomly selected observation points along the periphery of the four known clusters of the species in Kīpuka Kī. Throughout the study period, most observation points detected some flowering or fruiting. Seasons with the least amount of reproductive activity were fall 2006 with 73.7% of observation points fertile and summer 2007, when only 52.5% of points had flowers or fruit. The latter period was also a time of noticeable dieback of the vine with only 60% of observation points displaying live vine in July 2007. The vines recovered from this seasonal decline, and more than 90% of observation points had live vine from September 2007 until the study's end.

Inflorescences with male buds and flowers were most numerous in the fall, winter, and spring, while summer months were low points in the flowering cycle with male flowers seen at fewer than 10% of points (Fig. 36). The same pattern was seen with female inflorescences, with a decline in percentage of points showing female buds and flowers after June 2006 and after May 2007 (Fig. 37). Estimated abundance of female inflorescences was typically less than that of male inflorescences and never exceeded 100. Months that had a high proportion of observations with female flowers also displayed a high proportion of green fruit; in addition green fruit were present at most points for a month longer into summer 2006 (Fig. 38). Mature

fruit were persistent and were seen during most months (two months had no data). Peaks of mature fruit were not seen at consistent times throughout the years of the study but were noted in late summer 2006, spring 2007, and winter 2008 (Fig. 39).

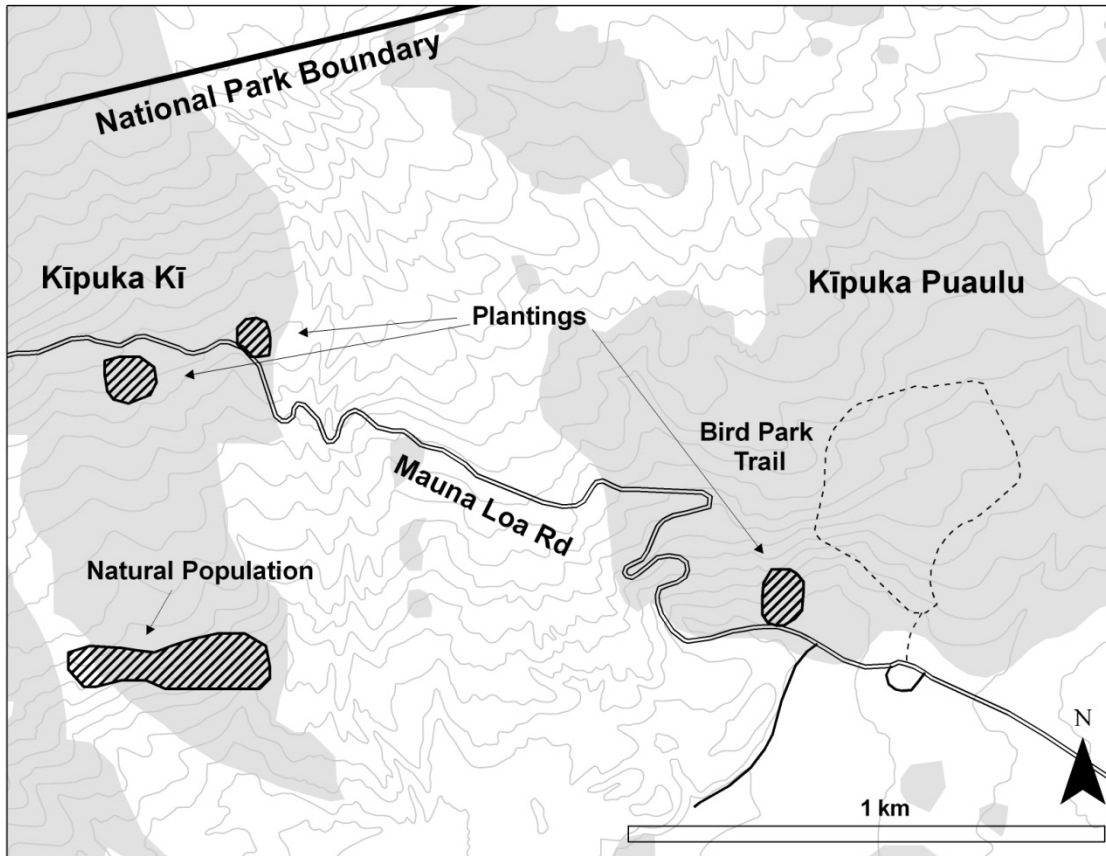


Figure 35. *Sicyos macrophyllus* natural population and plantings in Kīpuka Kī and Kīpuka Puaulu, HAVO, in 2008.

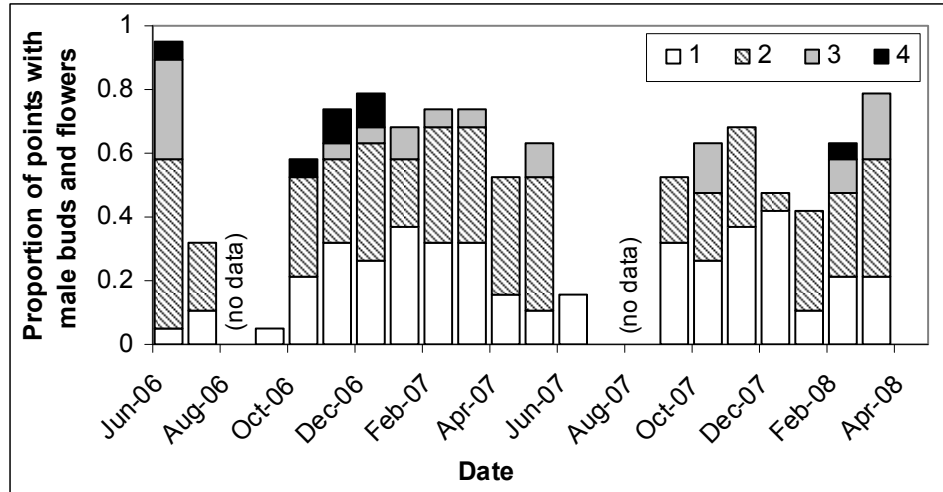


Figure 36. Estimated abundance of male inflorescences with buds and flowers in four categories for *Sicyos macrophyllus* in Kipuka Kī, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

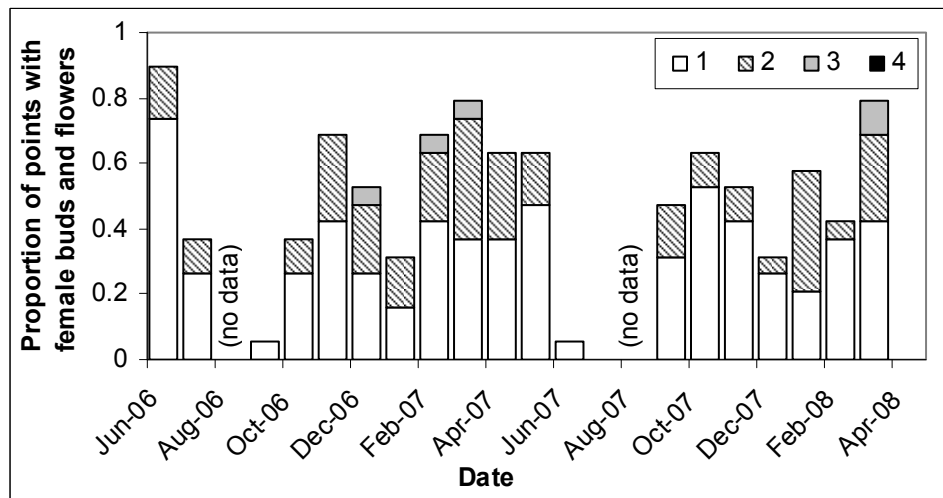


Figure 37. Estimated abundance of female inflorescences with buds and flowers in four categories for *Sicyos macrophyllus* in Kipuka Kī, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

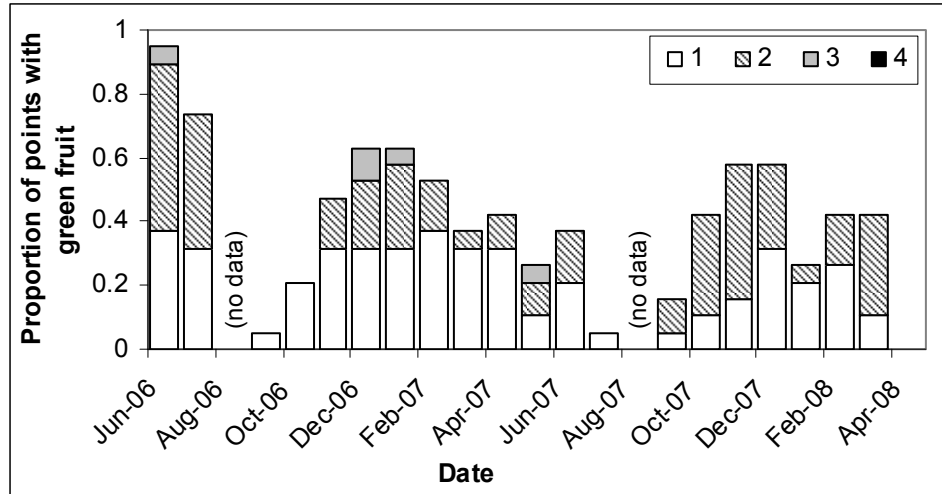


Figure 38. Estimated abundance of female inflorescences with green fruit in four categories for *Sicyos macrophyllus* in Kipuka Ki, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

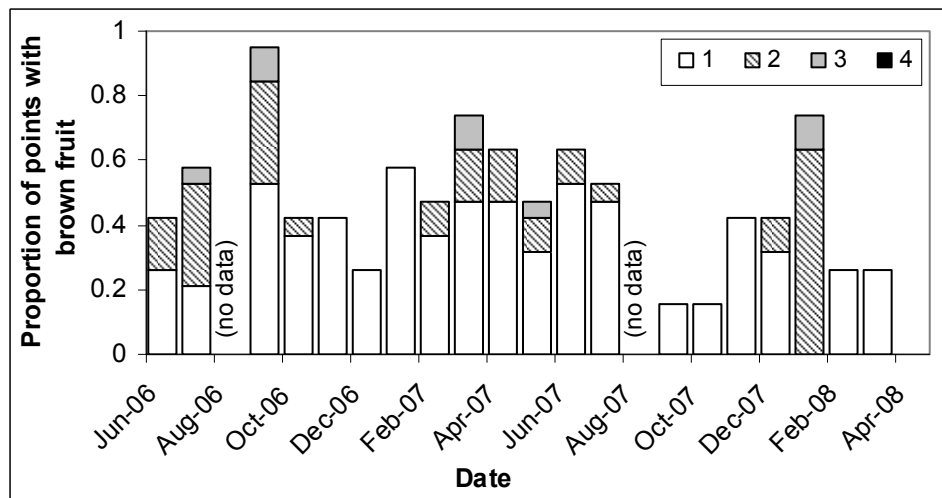


Figure 39. Estimated abundance of female inflorescences with mature brown fruit in four categories for *Sicyos macrophyllus* in Kipuka Ki, 2006-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

Success of Fruit Development

A group of tagged female inflorescences on randomly selected branches in winter and spring 2006 provided rates of successful transition from buds and flowers to large green fruit, and later to mature brown fruit. The rate of transition from individual buds and flowers to green fruit, large and essentially full size but still green and apparently immature, was 5% (n = 1017). These same buds and flowers successfully transitioned to brown, mature fruit at a rate of 2%. When inflorescences tagged bearing green fruit were added to this data set (n = 1159), the transition rate to mature fruit was 16%. Mean transition time from buds and flowers to immature green fruit was 1.2 months (SD = 1.0, n = 50), and the average time for green fruit to become brown, mature fruit was 0.5 month (SD = 1.0, n = 189). The mean number of buds and flowers per female inflorescence was 3.9 (SD = 5.5, n = 106), while the mean number of green and brown fruit per inflorescence was 4.4 (SD = 5.3, n = 148) and 1.3 (SD = 2.6, n = 94), respectively.

Pollination Studies

Floral visitation rates. Flowers of *S. macrophyllus* attracted a wide range of insects, including at least 24 species represented by the orders Hymenoptera, Diptera, Coleoptera, Hemiptera, Lepidoptera, Thysanoptera, and Collembola (Table 9). Hymenoptera and Diptera were most diverse, represented by six species each. Of the 24 insect species collected, 12 were observed visiting flowers during timed observation periods. Timed observations were dominated by Hymenoptera (81.4%) with the non-native honey bee (*Apis mellifera*) accounting for 73.1% of all flower visits. The second and third most abundant visitors were the alien golden paper wasp (*Polistes aurifer*) (7.5%) and the native seed bug (*Nysius* sp.) (7.3%), respectively. Thrips (Phlaeothripidae, status unknown) and non-native syrphid flies (*Allograptus exotica*) represented 5.7% and 3.4% of visits, respectively. The remaining eight species participated in less than 1.0% of all floral visits. Outside of the observation period, *Hylaeus difficilis* and *H. hula* were observed visiting flowers, documenting a new plant species record for these endemic yellow-faced bees (Daly and Magnacca 2003). Overall, approximately half of the insect visitor taxa were native, but their visits comprised only 13.9% of all visits.

Table 9. Visitation rates and duration of visits of insects on *Sicyos macrophyllus* flowers during timed observation intervals in Kipuka Ki, October to November 2006.

Floral Visitors	Status*	Total number of visits ¹	Mean visitation rate ² (SD) (n = 182)	Mean visit duration in sec ³ (SD) (n = # of visits)
HYMENOPTERA				
Apidae				
<i>Apis mellifera</i>	pur	361	1.02 (1.53)	6.4 (25.6)
Colletidae				
<i>Hylaeus difficilis</i>	end	<i>i</i>		
<i>Hylaeus hula</i>	end	<i>i</i>		
Formicidae				
<i>Cardiocondyla</i> sp.	adv	2	0.04 (0.46)	78.3 (36.2)
Ichneumonidae				
<i>Vulgichneumon diminutus</i>	adv	1	<0.01 (0.06)	180.0 (0.0)
Vespididae				
<i>Polistes aurifer</i>	adv	37	0.14 (0.71)	34.9 (33.5)
<i>Vespula pennsylvanica</i>	adv	<i>i</i>		
DIPTERA				
Calliphoridae				
<i>Chrysomya rufifacies</i>	adv	<i>i</i>		
<i>Pollenia rudis</i>	adv	<i>i</i>		
Undetermined sp. ⁴	?	1	0.01 (0.09)	2.0 (0.0)
Drosophilidae				
<i>Scaptomyza</i> sp.	end	1	<0.01 (0.06)	120.0 (0.0)
Syrphidae				
<i>Allograpta exotica</i>	adv	14	0.10 (0.41)	5.8 (7.9)
<i>Toxomerus marginatus</i>	adv			
Tachinidae				
<i>Trichopoda pennipes</i>	pur	<i>i</i>		
COLEOPTERA				
Coccinellidae				
<i>Scymnus loewii</i>	pur	<i>i</i>		
Nitidulidae				
<i>Prosopeus subaeneus</i>	end	3	0.02 (0.15)	460.0 (242.5)
HEMIPTERA				
Lygaeidae				
<i>Nysius</i> sp.	end	33	0.20 (0.77)	291.9 (249.9)
Miridae				
<i>Hylalopeplus pellucidus</i>	adv	<i>i</i>		
Nabidae				
<i>Nabis</i> sp.	end	1	<0.01 (0.06)	5.0 (0.0)
Pentatomidae				
<i>Eysarcolis ventralis</i>	end	<i>i</i>		

Table 9 (continued). Visitation rates and duration of visits of insects on *Sicyos macrophyllus* flowers during timed observation intervals in Kīpuka Kī, October to November 2006.

Floral Visitors	Status*	Total number of visits ¹	Mean visitation rate ² (SD) (n = 182)	Mean visit duration in sec ³ (SD) (n = # of visits)
LEPIDOPTERA				
Cosmopterigidae				
<i>Hyposmocoma</i> sp. 1	end	<i>i</i>		
<i>Hyposmocoma</i> sp. 2	end	<i>i</i>		
Crambidae				
<i>Mestolobes</i> sp. 1	end	<i>i</i>		
<i>Mestolobes</i> sp. 2	end	<i>i</i>		
THYSANOPTERA				
Phlaeothripidae				
Undetermined sp. ⁴	?	25	0.12 (0.39)	547.2 (30.3)
COLLEMBOLA				
Entomobryidae				
<i>Entomobrya laha</i>	end	4	0.02 (0.13)	504.0 (96.0)

* Status: adv = adventive or accidentally introduced; pur = purposely introduced; end = endemic to Hawaiian Islands; ? = unknown (Nishida 2002)

¹ Total number of visits to an individual flower by each observed insect species. *i* is an incidental observation outside the observational period.

² Rates represent the total number of flowers visited per total number of open flower observed per hour for each observation period. Mean visitation rate is based on the average across all observation periods.

³ Mean duration of a visit to an inflorescence based on individual visits independent of observation period.

⁴ Could not accurately identify to species level in the field.

Frequency of visits was dominated by the honey bee with a mean visitation rate of 1.02 visits/flower/hr (SD = 1.53, n = 182), followed by the seed bug (0.20 visits/flower/hr, SD = 0.77, n = 182), the golden paper wasp (0.14 visits/flower/hour, SD = 0.71, n = 182), and thrips (0.12 visits/flower/hr, SD = 0.39, n = 182). For the other visitor species, visitation was relatively infrequent (<0.10 visits/flower/hr). Of 361 visits by honey bees, 245 were to male flowers compared to 116 visits to female flowers. A significant difference was found between the mean visitation rates of honey bees at male (1.26 visits/flower/hr, SD = 34, n = 104) and female (0.72 visits/flower/hr, SD = 0.24, n = 86) flowers (Mann-Whitney U = 3660.0, p = 0.01). Collembola and thrips were generally present on an inflorescence for most of the duration of a period of observation while the visits of honey bees and paper wasps were relatively short (<15 sec.).

All visitors were observed exclusively feeding on the nectar of both male and female flowers. During visits, smaller insect taxa crawled into the corolla while larger taxa straddled the flower and probed with their proboscis. The diminutive size and shallow corolla cup of a *S. macrophyllus* flower enabled frequent contact of insect body parts with either the anthers or the stigma when smaller insect taxa entered and exited the corolla and when larger insect taxa crawled from flower to flower in an inflorescence.

Pollination experiment. No significant increase in the proportion of flowers per inflorescence that produced green fruit was achieved through artificial supplementation with pollen compared with naturally open-pollinated flowers (Mann-Whitney U = 510.5, $n_1 = 30$, $n_2 = 37$, $p = 0.56$) (Table 10). Fruit set was moderate in both treatments, only 14.6% set under open pollination and 17.9% through hand-pollination. On average, the number of green fruit produced per inflorescence was 1.2 (SD = 1.4) in the open pollination treatment compared to 1.4 (SD = 1.7) in the hand-pollination treatment.

Table 10. Mean number of *S. macrophyllus* flowers and green fruit set per inflorescence in the open- and hand-pollination treatments conducted in November 2006.

Treatment	Number of inflorescences	Mean number of flowers/ inflorescence (SD)	Mean number of green fruit/ inflorescence (SD)	Mean % of green fruit/ inflorescence (SD)
Open Pollination	30	7.6 (2.1)	1.2 (1.4)	14.6 (17.2)
Hand Pollination	37	7.1 (2.5)	1.4 (1.7)	17.9 (19.8)

Growth of plantings was very rapid soon after planting. In three vines measured for two months after planting in Kīpuka Puauulu in May 2006, average height increased from 10.3 cm to 28.6 cm in one week and to 46.0 cm in the second week. After a month, mean height of vines was 114.7cm, and this doubled to 246.7 cm after two months. Two of three vines were flowering within 1.5 months of planting, and young fruit were noted after two months. Three

vines planted in Kīpuka Kī grew from a mean height of 22 cm to 34.7 cm in four days and to 60.7 cm in 11 days (Thomas Belfield, pers. comm.).

Reproductive phenology 2007-2008. Almost all surviving *S. macrophyllus* vines planted in 2006 reproduced during the year of monitoring. Flowering and fruiting were noted on about half of plants in April 2007. Male inflorescences were present at low levels throughout the year, but were most abundant in spring and fall (Fig. 40). Female inflorescences with buds and flowers were also present throughout the year, but had a lower estimated abundance than male inflorescences. Most plants bore female flowers in fall 2007 (Fig. 41). Both green and brown mature fruit were observed on most plants during the year of monitoring. As was true for male and female flowers, infructescences were borne throughout the year. Green fruit as well as brown mature fruit were most abundant in the late spring and fall (Fig.s 42 and 43).

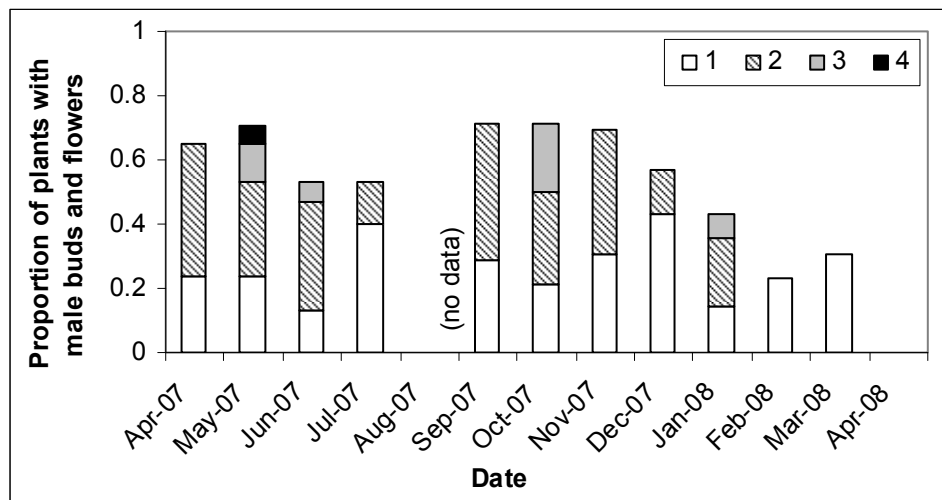


Figure 40. Estimated abundance of male inflorescences with buds and flowers in four categories for planted *Sicyos macrophyllus* in Kīpuka Kī and Kīpuka Puau, 2007-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

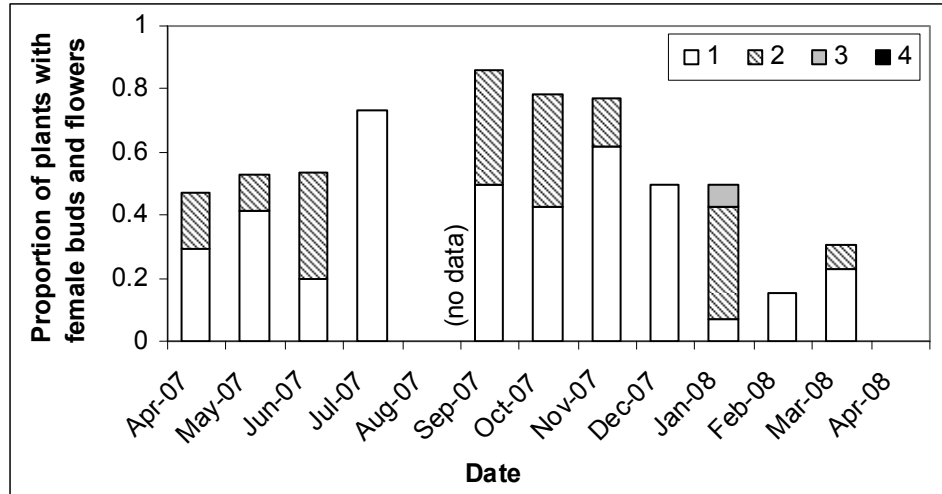


Figure 41. Estimated abundance of female inflorescences with buds and flowers in four categories for planted *Sicyos macrophyllus* in Kīpuka Kī and Kīpuka Puaulu, 2007-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

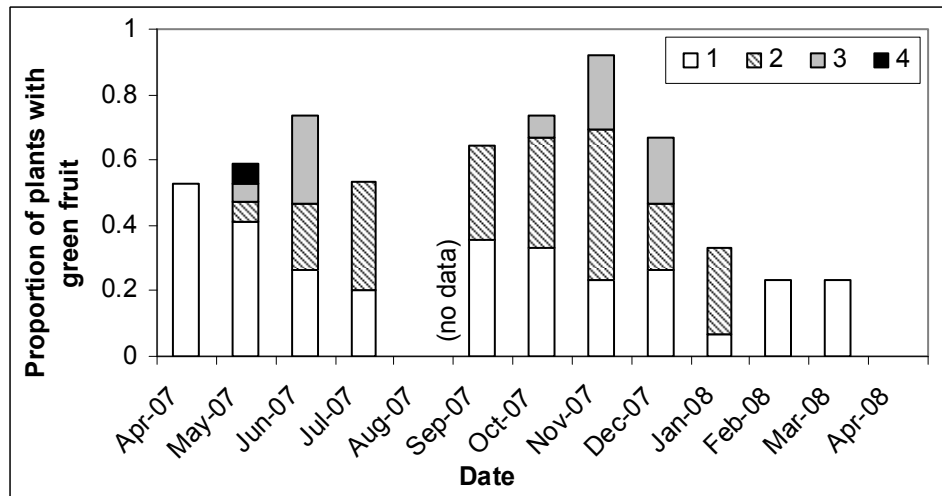


Figure 42. Estimated abundance of female inflorescences with green fruit in four categories for planted *Sicyos macrophyllus* in Kīpuka Kī and Kīpuka Puaulu, 2007-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

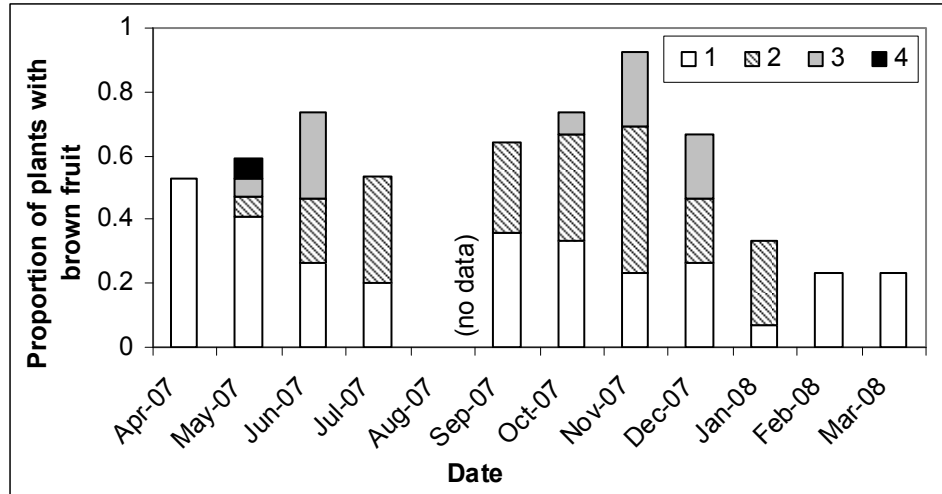


Figure 43. Estimated abundance of female inflorescences with brown fruit in four categories for planted *Sicyos macrophyllus* in Kīpuka Kī and Kīpuka Puaulu, 2007-2008 (categories: 1=1-10, 2=11-50, 3=51-100, 4=>100).

Seed Predation

Seed predation by rodents. To evaluate the occurrence of rodent seed predation we offered either large green or mature brown fruit in trays inside bait boxes to prevent loss due to wind, rain, or Kalij pheasants. In December 2006, four pairs of boxes were placed at each of the four natural *S. macrophyllus* sites in lower Kīpuka Kī, using 10 mature brown fruit in each offering. Overall 18% of seeds in open boxes were predated (leaving chewed husks) and 10% were missing after five months. In closed control boxes, 3% of fruit were missing. There was a significant difference between the number of disturbed (missing and predated) fruit in treatments and controls ($\chi^2 = 9.8$, $df = 1$, $p = 0.0017$). The mean number of missing or predated fruit was 2.8 (SD = 3.6) in open boxes and 0.3 (SD = 0.5) in closed controls.

In the second trial in February 2007, there were three replicate pairs of open and closed bait boxes, each with 10 large green fruit. Twenty-seven percent of fruit in open boxes were predated, and 20% were missing after three months. In closed control boxes, 7% of fruit were missing or withered and rotten at the end of the trial. This difference in the number of disturbed fruit appeared to be significant ($\chi^2 = 11.3$, $df = 1$, $p = 0.0008$). The mean number of

missing or predated fruit was 4.7 (SD = 5.0) in open boxes and 0.7 (SD = 0.6) in closed controls.

In the third trial in June 2007, we used 20 large green fruit in each offering with three pairs of treatments and controls. After four months, 18% of fruit was missing in open boxes and 2% were missing in closed boxes. There was no evidence of direct rodent predation (chewed fruit or piles of husks), but the number of missing fruit was significantly different in treatments and controls ($\chi^2 = 9.8$, $df = 1$, $p = 0.0017$). The mean number of missing fruit was 3.7 (SD = 4.6) in open boxes and 0.3 (SD = 0.6) in closed controls.

Seed Plots with and without Grass

Four months after planting, only one seed had germinated in one grass removal plot; no *Sicyos* seedlings were seen in any other plot. This seedling survived for two months and attained a height of 53 cm before it died. In addition to the *Sicyos*, we noted 21 volunteer *Coprosma rhynchocarpa* and 13 *Sophora chrysophylla* seedlings in cleared treatment plots, while no seedlings of native species were found in control plots. No other seedlings of *S. macrophyllus* appeared in plots during a year of monthly monitoring and none was present when the site was revisited in February 2009.

Seeds and Seed Germination

Soil seed bank. The soil seed bank was sampled three times in different seasons. In June 2006, we collected ten cores from beneath randomly selected branches at Site A, five cores from Site B, and six from Site C. We counted 50 fruit and 52 fragments in all cores combined. Twenty-three fruit were whole (22.5%) and 27 (26%) were obviously predated by rodents with gnaw marks on the husks and the seeds consumed. The 52 fragments (51% of the total fruit) almost certainly represent remnants of predated fruit. Most of the material recovered was from Site A, and no seeds or fragments were found at Site C.

In November 2007 and February 2009, we repeated the soil coring, with an equal number of cores (five) from each site (A, B, and C). In the 2007 trial there were seven whole fruit, five predated fruit, and no fragments in all cores combined. All but one predated fruit/seed came from Site A. This amounted to 58% predation in recovered fruit/seeds. In February 2009 there were four whole fruit or seeds and 11 seeds predated by rodents (73%

predation) in cores at site A and no *S. macrophyllus* seeds or remnants in cores from sites B or C. Several of the whole seeds were completely free of the enclosing inner fruit wall. Whole seeds of common tree species, such as *A. koa*, *S. saponaria*, and *S. chrysophylla*, were also recovered from cores at the three sites.

Seed germination in the greenhouse. After some preliminary trials to establish seed treatment techniques and potting mixtures, there were six germination trials with *S. macrophyllus* seeds in 2006 and 2007. Germination rates were extremely variable in 2006, ranging from 0 to 42.4% in three trials. In the first trial, fruit were peeled or nicked with a clipper, and germination rates ranged from 16 to 56% in five flats with a mean of 42.4%. In the second trial, no special treatment was given to seeds still enclosed in the fruit wall before they were planted; no germination was observed in this trial, and seeds/fruit rotted after sowing. The third trial in 2006 used fruit nicked with a clipper to the enclosed seed, but most seeds rotted within a month of planting. The mean germination rate for this trial was 5.7%. Germination rates were more consistent in 2007, when all seeds were peeled out of the thick woody fruit husk or pericarp. The overall mean germination in 2007 was 27.1%, ranging from 21.3 to 32% in the three trials. In 2007, seeds began to germinate within 19 days of sowing (Appendix II, Table 4).

Seed viability test. Mature brown fruit of *S. macrophyllus* were collected from four separate plants, all of which were outplantings in Kīpuka Kī and Kīpuka Puaulu. Three of the plantings were in Kī and one was in Puaulu. Twenty seeds from each kīpuka were used in the viability test. None of the fruit floated during imbibing. After bisecting, all 20 seeds from Kī appeared healthy, whereas five from the Puaulu plant appeared to have a fungal infection, which had caused severe deterioration of the endosperm.

All of the Kīpuka Kī seeds were tested. One was a strongly positive, 11 were positive, seven were weakly positive, and one was negative. The negative seed appeared healthy but showed no staining. Only the 15 seeds from Puaulu that appeared healthy were tested. Of these, seven were strongly positive, seven were positive, and one was weakly positive.

Summary of Limiting Factors for Five Species Investigated in this Study

Each species appeared to have more than one possible limiting factor in this study of five rare plants in the mesic forests of HAVO. A summary of the results of stand structure

analysis, the patterns of reproductive phenology, pollination studies, and details on potential limiting factors we addressed in this study for each species are presented in Table 11.

Hibiscadelphus giffardianus is a re-introduced population with a high survival rate (> 70%). The stand structure of *M. hawaiiensis* and *M. zahlbruckneri* indicated declining populations since trees were visited 15 years ago. The population of *Z. dipetalum*, which includes trees planted at least 30 years ago, has also declined. Four groups of *S. macrophyllus* vines have persisted in place for more than eight years. All four tree species had continuous patterns of flower and fruit phenology, although seasonal peaks of abundance were observed. The vine *S. macrophyllus* had an annual or subannual pattern of reproductive phenology. Successful transition of flowers to fruit was high for *Z. dipetalum* (47%), moderate for *M. hawaiiensis* (18%) and *S. macrophyllus* (5%), low for *H. giffardianus* (1.8%), and extremely low for *M. zahlbruckneri* (0.5%). High percentage viability was demonstrated for seeds of all five species (>95% weakly to strongly positive). Greenhouse germination rates were high for *Z. dipetalum* seeds (55.3-56.7%), variable and moderate for *H. giffardianus* (9-30.9%) and *S. macrophyllus* (21-32% in 2007), and low for *M. hawaiiensis* (7.1-11.1%) and *M. zahlbruckneri* (6.7%). No appreciable intact soil seed bank was detected for any species other than *M. hawaiiensis* and *S. macrophyllus*. Field seeding in grass-free plots resulted in the establishment of few seedlings of *H. giffardianus* and one *S. macrophyllus*. Plantings of greenhouse-grown seedlings of *Z. dipetalum* failed in both grass-dominated and grass-free sites in Kīpuka Puʻaʻulu.

For *H. giffardianus* there was no significant difference in fruit production inside or outside rodent exclosures. Episodic bark-stripping and flower feeding by rats was detected, and seed offerings were quickly consumed by rodents. No evidence of seed predation or sapling damage by rats was demonstrated for *Z. dipetalum*, but there was a significant difference in survival of natural seedlings inside and outside Kalij pheasant exclosures. Rodent seed predation was noted in the soil seed bank of all species except *Z. dipetalum*. Seed offerings of *S. macrophyllus* exposed to rodents showed significantly greater loss than did inaccessible controls. Rodent predation of capsules on trees was episodically high in *M. hawaiiensis* (54-57%) but was rarely observed in *M. zahlbruckneri*. No evidence of predation was noted at seed offerings of *M. hawaiiensis*. Predation by caterpillars of native moths (*Prays* spp.) led to damage of a high percentage of sampled capsules of both *M. hawaiiensis* (25%) and *M. zahlbruckneri* (72-100%).

Floral visitors were observed at three rare plant species, and hand-pollination experiments were carried out on two of them. Bird visitors to *H. giffardianus* flowers were primarily *Hemignathus virens virens* (Hawaiian Amakihi), which robbed nectar from the base of flowers, and less often *Zosterops japonicus* (Japanese White-eye), which typically inserted their heads into the top of flowers. Insect visitors to *H. giffardianus* were *Scaptomyza palmae* fruit flies, *Prosopius subaeneus* sap beetles, and *Apis mellifera*, the honey bee. Pollen washing documented low levels of *H. giffardianus* pollen on fruit flies and sap beetles. Sample flowers were cross-pollinated by hand and compared with treatments of induced self-pollination, open-pollination, and natural self-pollination of bagged flowers. Cross-pollinated flowers had significantly greater numbers of germinated pollen grains on stigmas than did those of the induced self-pollinated flowers. Pollen was detected on open-pollinated flowers, but none was found on bagged flowers. Using fluorescence microscopy, no pollen tubes were detected in the flower styles in any treatment.

The only visitor to flowers of *M. hawaiiensis* during timed observations was *Allograpta exotica*, an introduced syrphid fly. The floral visitation rate of the fly was very low (0.17 visits/flower/hour), and more male than female flowers were visited. Twelve insect species visited *S. macrophyllus* flowers during timed observations, and another 12 species were incidental visitors. *Apis mellifera* accounted for 73.1% of all flower visits. No significant increase in the proportion of flowers that produced fruit was achieved through artificial supplementation with pollen when compared with open-pollinated flowers of *S. macrophyllus*.

Table 11. Potential limiting factors of five rare plant species in Kīpuka Puauulu and Kīpuka Kī investigated in this study.

Factor	<i>Hibiscadelphus giffardianus</i>	<i>Melicope hawaiiensis</i>	<i>Melicope zahlbruckneri</i>	<i>Sicyos macrophyllus</i>	<i>Zanthoxylum dipetalum</i>
Stand structure	Planted	Declining	Declining	Unknown	Declining
Flower/fruit phenology	Continuous	Continuous	Continuous	Annual	Continuous
Fruit production	Low	High	Low	High	High
% Flower to green fruit	1.8%	18%	0.5%	5%	47%
Natural seedlings	One	Few	None	One	Many
Seed germination (greenhouse)	9-31%	7-11%	0-6.7%	21-32% (2007)	55-57%
Seed viability test positive to strong	100%	65%	100%	74%	95%
Soil seed bank	No	Yes	No	Yes	No (small)
Rat predation of seed in soil	Yes	Yes	Yes	Yes	No
Rat predation of fresh seeds	Yes (83%)	No	n/a ¹	Yes (7-28%)	No
Rat bark stripping	Yes	No	No	No	Few trees
Rat predation of fruit on plant	Yes (10%)	Yes (54-57%)	No	No	No
Insect fruit predation	No	Yes (25%)	Yes (72-100%)	No	No
Seed germination/ sdlg survival in grass	n/a	n/a	n/a	No	No
Kalij damaged sdlgs	No	n/a	n/a	No	Yes
Floral visitors identified	Yes	Yes	n/a	Yes	n/a
Pollen viability	75.4%	n/a	n/a	n/a	n/a
Stigma receptivity	100%	n/a	n/a	n/a	n/a
Pollen limited or self-incompatible	Yes	n/a	n/a	No	n/a

¹ n/a, not applicable, factor not examined or tested.

DISCUSSION

Population Structure, Growth, and Survival 1993-2008

Comparison of the current population structures of three of the four tree species studied with those of 15 years ago revealed that all have declining populations with low or non-existent seedling recruitment. Populations of *M. hawaiiensis*, *M. zahbruckneri*, and *Z. dipetalum* have all decreased in HAVO since previous surveys. Because all the *Hibiscadelphus giffardianus* trees of HAVO have been planted, we have no data on natural population structure of that species. Most *H. giffardianus* trees in the two kīpuka are the result of a planting effort in 2000-2001, and a few trees remain from an earlier planting in 1995-1997. Survival of the original plantings made in 1951-1964 was apparently not high, as 28 were planted in Kipuka Puaulu (Morris unpublished), nine were present 11 years ago (U. S. Fish and Wildlife Service 1997a), and only seven remain. At least two of the oldest trees have died in the last 10 years and others have toppled but survived. *Hibiscadelphus* trees may not be inherently long-lived, because they are fast-growing, soft-wooded trees. Nothing is known about longevity in this species (U. S. Fish and Wildlife Service 1997a).

It is unclear whether old *H. giffardianus* trees in Kīpuka Puaulu are one or two generations removed from the original single tree. Fagerlund (1944) wrote that seeds of the original tree were planted by William Giffard in Volcano Village, and the resulting tree was bruised to make cuttings that were planted on Keauhou Ranch adjacent to the Park. There is also the possibility that the Giffard tree was a clone of the original (Baker 1980). Presumably, the oldest living trees in Kīpuka Puaulu are derived from the ranch tree (Baker and Allen 1977), but whether they are clones or seedlings is not documented (Morris unpublished). The question of clonality is relevant to the success or failure of sexual reproduction in some species (Warburton *et al.* 2000).

By contrast with earlier restoration attempts, recent *Hibiscadelphus* plantings in the two kīpuka have been extremely successful with almost three quarters of trees planted surviving more than eight years. The higher mortality rate in Kīpuka Puaulu when compared with Kīpuka Kī may be due to the dense understory present in Puaulu, which results in lower light conditions for rapidly-growing trees. *Hibiscadelphus giffardianus* trees are clearly fast-growing, and survivors achieved heights of nearly eight m and diameters of approximately six cm within just

seven to eight years. Reproduction also begins at a relatively early age, since most young trees in the current study were observed producing buds and flowers.

Unlike *H. giffardianus*, the HAVO populations of *Melicope hawaiiensis* and *M. zahlbruckneri* are largely natural; HAVO Natural Resources Management Division records indicate that 20 *M. hawaiiensis* seedlings were planted in Kīpuka Puauulu in 1978-1980, and only six remained alive in 1983 (Zimmer unpublished). One mapped planting on the eastern side of the loop trail corresponds with an existing tree, which may represent a successful planting of this species; the specific localities of the other former plantings are unknown. Two *M. zahlbruckneri* planted in 1978 in Kīpuka Puauulu died according to monitoring notes, but a third planted in 1979 apparently lived (Zimmer unpublished). The described planting site corresponds with the one small plant we found near the trail. If this small tree is indeed planted, it has not achieved a height greater than a meter in almost 30 years.

Compared with *M. zahlbruckneri*, the population of *M. hawaiiensis* in HAVO appeared robust when first inventoried in 1993, when a third of known trees were of small diameter and presumably young. The presence of both young and old trees indicated a relatively stable and reproducing population. The change in the population structure displayed by 2008 indicated a loss of mature trees and a slow increase in diameter of trees in middle size classes. It is doubtful whether low recruitment since 1993 will be sufficient to maintain the population as old trees become senescent and die. Certainly the Park population has decreased in the last 15 years despite protection of its habitat from feral ungulates and the most invasive alien plant species (Tunison and Stone 1992). Despite the population decline, HAVO may have the most populous and best protected site for this species on the four Hawaiian Islands on which it occurs (Stone 1969). The next largest known population of *M. hawaiiensis* on Hawai`i Island is that of Pu`uanahulu Game Management Area, where 30 individuals were observed more than ten years ago (Shaw *et al.* 1997). Nothing is known about growth rates in the Park's *Melicope* species. Extrapolating from the mean diameter growth of surviving trees in the current study, the annual growth rate of *M. hawaiiensis* is half that reported for all common tree species on the International Biological Program Mauna Loa transect (Lamoureux *et al.* 1981).

The endangered *M. zahlbruckneri* population is declining within Kīpuka Puauulu, as 46% of known trees died or disappeared between 1993 and 2008. The current stand structure is that of a senescent population which is not reproducing (Barbour *et al.* 1980, Mehrhoff 1989);

most documented mortality was that of trees in large diameter classes, which presumably died after reaching old age. The presence of small trees in 1993 was encouraging and indicated some level of natural reproduction, although with so little known of growth rates it is uncertain how young small trees really were. This population size and structure was not maintained, and by 2008, the only small tree was a trailside plant that had been repeatedly damaged and had scarcely grown in 15 years (and may represent the only surviving planted tree). The combination of high adult mortality and failure of recruitment may indicate the population is in a latter stage of decline, and only the longevity of adult trees has prevented the previous extirpation of the species from HAVO. The extrapolated annual growth rate of trees surviving between 1993 and 2008 was half that reported for common tree species on Mauna Loa (Lamoureux *et al.* 1981) and similar to the calculated growth rate of *M. hawaiiensis*. *Melicope zahlbruckneri* remains one of the most critically endangered plant species of HAVO, and focused efforts may be required to prevent loss of the species. Certainly the situation is far different from that of 1913, when Joseph Rock, the discoverer and author of the species, referred to the tree as “quite plentiful” in Kīpuka Puaulu (Rock 1913).

Zanthoxylum dipetalum var. *dipetalum* has been previously propagated and planted in Kīpuka Puaulu (Morris unpublished, Zimmer unpublished). The size class structure of the species in Kīpuka Puaulu in 1993 was characteristic of a growing population capable of maintaining itself (Barbour *et al.* 1980), but this population structure was deceptive, as it was based on a pronounced small-diameter class that included trees planted in the 1970s. HAVO records indicate that 50 *Z. dipetalum* var. *dipetalum* trees (at the time called var. *geminicarpum*, see Rock [1913]) were planted in Kīpuka Puaulu between 1974 and 1978, and at least 14 survived until 1981 (Zimmer unpublished). This mortality rate of 72% is similar to the 60% loss we observed in seedlings recently planted in Kīpuka Kī.

It is difficult to determine the number of *Z. dipetalum* trees currently growing unassisted within Kīpuka Puaulu, but it is possible that as much as a third of the standing population is natural. Based on the current and past (1993) diameter measurements and the apparent slow growth of the species, it is almost certain that at least five live trees (and one dead) with diameters >25 cm are of natural origin. Another three trees >16 cm in diameter may be natural components of the kīpuka, although they may also be survivors of an planting of 11 trees in 1946-48 (Morris unpublished). This is a larger number of natural trees than the two

trees observed in the kīpuka by Joseph Rock in 1911 (Rock 1913). Subsequent to Rock's visits, the kīpuka was fenced against cattle (Apple 1954), and native trees (including *Zanthoxylum*) may have had success recruiting new trees during the >60 years prior to the first systematic survey of rare trees in 1992-93. Natural seedlings were reported from beneath two large trees in the 1960s (Morris unpublished). Kalij pheasants, identified in the present study as potential seedling predators, did not appear in HAVO until the late 1970s (Lewin and Lewin 1984).

We discovered one relatively recent *Z. dipetalum* recruit that was far too small to have been planted 30 years ago, and two additional small trees growing directly beneath a large female represent natural recruitment at some time in the past. The remaining 42 trees likely represent both planted material and natural recruitment. One tree measuring 4.3 cm in diameter bore a tag dated 1974. Natural reproduction and recruitment of trees has probably been very low over the last several decades; only the group of nine trees with diameters less than 2.5 cm (excluding trees with partly dead stems) and the three young plants discussed above are likely to include natural recruits from the last 35 years.

The vine *Sicyos macrophyllus* is of a growth form not amenable to evaluation of stand structure. The presence of this species at multiple sites in Kīpuka Kī was only confirmed in 2000; previously an observer had collected a specimen from one vine in 1996 (Lyman Abbott, sn, HAVO Herbarium). Since there are no known extant populations near Kīpuka Kī (other than the population far away at Kīpuka Mauna`iū) from which *Sicyos* could have moved into the kīpuka, it appears that the species has persisted there despite its use as a cattle pasture for about 100 years until 1948 (Apple 1954, Baldwin and Fagerlund 1943) and the presence of feral pigs and goats until the 1980s (Katahira and Stone 1982, Katahira *et al.* 1993). The annual dieback and seasonal nature of growth in the genus *Sicyos* (Wagner *et al.* 1999) may have made the Park population less conspicuous to observers in its mesic forest habitat. *Sicyos macrophyllus* appears to be a perennial. While we cannot determine how many individuals are represented in the Kīpuka Kī population, there are four groups each separated by approximately 100 m, and we have observed at least one natural seedling during our monitoring. The rapid growth of the vine would permit seedlings to integrate with adult vines in the intervals between monthly observations.

Patterns of Reproductive Phenology

It is notable that the phenological pattern we observed in four of five monitored species was characterized by continual flower and fruit production throughout the year. While peaks in bud, flower, and fruit production were detected, some flowers and fruit were present year-round on all species except *Sicyos macrophyllus*. This continual flowering pattern is typical of tropical forest communities (Newstrom *et al.* 1994), but is uncommon in individual tropical tree species (van Schaik *et al.* 1993) or in neotropical wet and dry forest treelets and shrubs (Opler *et al.* 1980). The continual presence of seed-bearing fruit throughout the year may have implications for the impacts of rodent predation on seeds that we observed with three of the four monitored tree species. Since rats are opportunistic feeders known to vary their diets with seasonal abundance (Cole *et al.* 2000, Sugihara 1997), rats that learn to feed on continuously available fruit may take a disproportionate amount of the seed crop of favored species.

Hibiscadelphus giffardianus showed a pattern of continual flowering throughout the year; the pattern was particularly pronounced for bud production, where almost all trees bore buds in every month. Fruit capsules were present on more than a quarter of trees throughout the year, although this is partly due to the persistence of mature capsules rather than continual production. Even though a continual pattern of flowering was displayed, there were pronounced summer peaks in flower production observed over two years. Peaks in flowering associated with strength of the sun have been observed in other tropical forests (van Schaik *et al.* 1993).

The continual pattern of flower production was also seen in *Melicope hawaiiensis*, although there was a gap in flower presence in fall 2006. Seasonal peaks in buds and flowers were observed during winter and spring months for this species. Fruit production was not continual, but there was only a short winter break in fruit-bearing. Some fruit was present on a low percentage of trees throughout almost all the year. The pattern of bud and flower production in *M. zahlbruckneri* was similar to that of its more common congener. Fruit production was very low, and the proportion of trees bearing fruit was typically less than 20%, but some capsules were present on these few trees year-round. Berlin *et al.* (2000) detected year-round fruit production with a seasonal peak in a related common species on Maui (*M. clusiifolia*).

Zanthoxylum dipetalum var. *dipetalum*, a member of the same family as *Melicope* spp., had flowering peaks less pronounced than those of *Melicope*, with flowers present in fall, winter, and spring. Fruit capsules were observed throughout the year; green immature fruit were very persistent and required several months to ripen. Small peaks in fruit production were noted in spring months. Lack of seasonality in fruiting is sometimes associated with small seed size (Sun *et al.* 1996); this is not the case with *Z. dipetalum*, which had the largest size seeds of the five species studied.

Sicyos macrophyllus showed the most seasonal pattern of all the mesic forest species we studied. Rather than the continual pattern of flowering seen in the four tree species of the current study, this vine showed an annual or subannual pattern of flowering (Newstrom *et al.* 1994). Male and female flower production peaked twice a year in fall and spring, but flowering was also seen throughout the intervening winter months. During summer months plants produced virtually no buds or flowers, although this observed low period is exacerbated by a gap in data collection in August for both 2006 and 2007. Seasonal massing of flowers at one or few times a year may increase attractiveness to pollinators and be timed to population peaks of pollinating insects (van Schaik *et al.* 1993). Male flowers were produced in greater abundance slightly earlier than female flowers; it is unknown if this encouraged out-crossing in this monoecious species. Green fruit production was seasonal, showing an annual pattern with a peak in winter and no or little fruit production in late summer or early fall. The winter production of green fruit that ripen in the winter and spring may be an adaptation to avoid seed damage by pests more abundant in summer; a pattern of more severe fruit damage in summer has been noted for many temperate plant species (Rathcke and Lacey 1985). Young planted vines showed a distinct subannual pattern of flower and green fruit production. A continual pattern of brown fruit presence was observed, probably because of the persistence of these mature structures on vines even after leaves wilted and stems dried out.

Relatively few phenological studies have been carried out on Hawaiian plants, with the exception of forest dominants, such as *Metrosideros polymorpha* (Porter 1973, Carpenter and MacMillen 1973, Carpenter 1976), *Sophora chrysophylla* (Pratt *et al.* 1997, Banko *et al.* 2002), and *Acacia koa* (Lanner 1970). Berlin *et al.* 2000 studied the flowering and fruiting phenology of *Metrosideros* and nine other native plant species in a cloud forest on Maui and found an annual pattern of flower production in all species studied. At least two species studied by Berlin

et al. (*Cheirodendron trigynum*, ōlapa, and *Melicope clusiifolia*, alani) had continual production of fruit over three years.

Sophora chrysophylla, the dominant tree of subalpine forests on Mauna Kea and the primary food plant of the endangered Palila (*Loxioides ballou*), has been the subject of long-term studies and demonstrates an annual phenological pattern of flowering and fruiting with strong seasonality of peak flower and pod production (Pratt *et al.* 1997, Banko *et al.* 2002, van Riper 1980). *Acacia koa*, a forest dominant in montane dry and mesic forests on Hawai`i, was studied on the Mauna Loa Strip within HAVO where flowering and growth in height were found to be strongly seasonal (Lanner 1970).

One of the few plant phenological studies that did not involve birds followed a suite of 12 native tree and shrub species on an elevational transect in HAVO for five years and found annual flowering patterns in all species studied. Typically peak flowering occurred either in winter wet months or the warm and dry period of late summer. For the widespread species *Acacia koa*, flowering was synchronized at approximately the same season respective of elevation and moisture regime. Among the species studied, only *Sophora chrysophylla* showed a continual flowering pattern at both a low and high elevation site (Lamoureux 1973, Lamoureux *et al.* 1981).

There appears to be a disparity in the phenological pattern of four of the five rare plant species in mesic kīpuka forests when compared to patterns reported for more common tree species in Hawai`i. Most of the studies of more common species involved larger sample sizes and multiple study sites; several were of duration of more than five years. Longer-term phenological studies of our target species may be required to determine whether the observed continual flowering and fruiting patterns are inherent in the rare plant species or related to some short-term fluctuation in the climate patterns at the mesic forest study sites.

Pollination

Hibiscadelphus giffardianus flowers were visited by the native Hawai`i `Amakihi and the non-native Japanese White-eye. Hawai`i `Amakihi was the dominant visitor with 65% of total floral visits, and the Japanese White-eye made up the other 35% of visits. These results notably differ from Baker and Allen (unpublished) who also conducted floral visitor observations on *H. giffardianus* in Kīpuka Puauulu and observed Japanese White-eye as the primary floral

visiting species. In this older study, Japanese White-eyes accounted for 97% of all visits, and Hawai`i `Amakihi and `Apapane (*Himatione sanguinea*) composed the other 3%. It is uncertain why this discrepancy exists, and there is no evidence indicating that the relative abundance of the two species has changed in the last 30 years. In a recent analysis of forest bird population trends on windward Mauna Loa between 1977 and 2003, `Amakihi were found to be slightly decreasing, and Japanese White-eyes showed a significant increase in the study area (Gorresen *et al.* 2005). In a study of bird populations over three years in Kīpuka Puauulu and Kīpuka Kī, Japanese White-eyes had much higher frequency at count stations in the two kīpuka (100%) than did Hawai`i `Amakihi (0-13%) (Sarr *et al.* unpublished). Differences in species composition of floral visitors in the two studies is more likely explained by differences in the time of year of observations and the varying abundance of flower resources throughout the seasons. Baker and Allen carried out their study during one week in July and August, and our observations were made during several seasons.

Hawai`i `Amakihi and Japanese White-eye are not adapted to the floral morphology of *H. giffardianus*. The length of the floral tube exceeds their bill length hindering legitimate extraction of nectar available at the base of the corolla. Instead, both birds obtain nectar by inserting their bills into tears through the corolla above the calyx. It is uncertain which species is responsible for perforating the floral tissue, but as the dominant nectar-robber, it is possible these tears are primarily made by the larger `Amakihi, and Japanese White-eye are secondary nectar-robbers using preexisting tears. This foraging by nectar-robbing does not result in pollination as the bodies of these birds fail to come into contact with the plant's sexual organs. Even though nectar-robbers may also be pollinators (Navarro 2000), most studies have shown that robbers deter legitimate pollinators by interference competition or reducing the availability of nectar resources (Gill *et al.* 1982, Roubik *et al.* 1985). Two possible original pollinators, Hawai`i `Akialoa (*Hemignathus obscurus obscurus*) and Hawai`i Mamo (*Drepanis pacifica*), are now extinct (American Ornithologists' Union 1983) and the only remaining long-billed bird, the I`iwi (*Vestiaria coccinea*), has become relatively rare in the two kīpuka and may be declining in the region (Gorresen *et al.* 2005).

Floral visits by Japanese White-eye primarily involved inserting their head down into the corolla tube from the top, rather than from the side (robbing). This is presumed to be a form of insectivorous feeding on flower-inhabiting insects. This form of foraging behavior may lead

to cross-pollination as the crown of the birds' heads incidentally contacts anthers in search of insects. Future studies of pollen load analyses on crown plumage of mist-netted Japanese White-eyes would be needed to confirm effective pollination.

Fruit flies and a single honey bee were the only insect visitors seen during the timed observations of *Hibiscadelphus* flowers. Although fruit flies prevailed as the primary insect floral visitor, they did not appear to collect floral resources, but instead used flowers as a breeding site. The honey bee, in its single visit, was observed crawling down the length of the corolla tube and presumably fed on nectar. Outside of the observation period, incidental observations were made of Kamehameha butterflies feeding on nectar and numerous sap beetles residing inside the corolla tube, where they were probably feeding on yeasts growing in the nectar (Lachance *et al.* 2005).

Pollen load analyses of insects collected near *H. giffardianus* indicated that the sap beetles and fruit flies were carriers of pollen of this species. However, the large proportion of pollen from other plant species in their pollen loads suggested that neither insect taxa restricted their visits to the flowers of *H. giffardianus*. Despite the abundance of the insects, the rather low number of *H. giffardianus* pollen grains carried on their bodies likely reduces their ability to pollinate a large proportion of flowers. More research is needed to track the distances travelled by these insect species to determine their potential for cross-pollinating plants.

In the hand pollination tests for self-compatibility, pollen deposition provided evidence for both avoidance of selfing in *H. giffardianus* flowers and the natural occurrence of cross-pollination by floral visitors. In *H. giffardianus* flowers, the stigma is spatially separated from the anthers. Plants will vary the spatial distribution of male and female organs within hermaphroditic flowers to reduce the likelihood of inbreeding through intrafloral self-fertilization (Charlesworth and Charlesworth 1987). The absence of pollen on the stigmas of bagged flowers with no manipulation demonstrated the effectiveness of this adaptation. Therefore, the presence of pollen on the stigmas of the open-pollinated flowers with no manipulation indicates deposition of pollen by a floral visitor. In addition, the similarly comparable germinability rates between the open-pollinated and cross-pollinated flowers, implies that the pollen deposited on the stigmas by floral visitors in the open-pollinated flowers is not self pollen, but outcross pollen.

Overall, it appears the reproductive success of *H. giffardianus* is especially limited. Attempts made to increase fruit set with supplemental pollen in the pollen limitation study, resulted in no increase of reproductive output. Low pollen viability or stigma receptivity was not the reason for this lack of reproductive success. Tests of male and female fertility demonstrated a high degree of pollen viability and receptive stigmas throughout all stages of flower development. Instead, fluorescence microscopic examination revealed two pre-zygotic factors inhibiting seed set. First, there was an inability of pollen tubes to penetrate the stigmatic surface and enter the style to deliver sperm to the ovule. Second, the average number of pollen grains with tubes for the cross-pollinated flowers was almost double that of the self-pollinated flowers. Angiosperms frequently have self-incompatibility mechanisms encoded into their genotypes whereby stigmas of flowers are more likely to reject their own or closely related pollen to avoid inbreeding. Two basic mechanisms of self-incompatibility are sporophytic self-incompatibility where pollen is rejected at the surface of the stigma and gametophytic self-incompatibility where a pollen tube starts to grow, but then its growth is arrested before it reaches the ovaries (Newbigin *et al.* 1993). The observed stunted pollen tubes and higher pollen germination rates in the cross-pollinated flowers suggests a sporophytic self-incompatibility mechanism evolved in *H. giffardianus* to avoid self-fertilization and promote outbreeding.

In small populations where the availability of mates is limited, self incompatibility further limits the number of potential mates due to restrictions in the breeding system. Individuals of self-incompatible species will typically recognize and likely reject not only pollen from themselves, but also the majority of pollen from grandparents, parents, siblings, and offspring (Newbigin *et al.* 1993). This genetic limitation may be a factor leading to low seed set in *H. giffardianus* because all the individuals that occur today are direct descendents of a single plant.

During this study, flower visitation in *M. hawaiiensis* was dominated by the non-native syrphid fly *A. exotica*, which displayed a strong preference for male flowers. The reason for this preference is uncertain. Adult syrphids are flower specialists that require nectar for energy and pollen for proper egg development (Schneider 1969). The small narrow corolla opening of the young male flowers made it difficult to discern which of these floral resources was being harvested. Either *A. exotica* were particularly attracted to the pollen or there may have been more nectar available in male flowers. Increased nectar productivity in male flowers has been

linked with pollinator preference for male flowers in a number of systems (Bell *et al.* 1984, Devlin and Stephenson 1985). Further studies are needed to compare levels of nectar production with respect to *M. hawaiiensis* flower sex.

Melicope hawaiiensis, as a monoecious plant, requires cross-pollination by floral visitors for fruit production. Although this study demonstrated low visitation rates by *A. exotica*, the moderate fruit set (Fig. 17 and 18) observed in *M. hawaiiensis* suggests *A. exotica* are providing an important cross-pollinating service. Visits by the native koa butterfly (*U. blackburni*) and sap beetle (*P. subaeneus*) were rare, indicating there may have been a loss of a more common native pollinator and/or *A. exotica* may be hindering native insect visitation through floral resource competition.

The open morphology of *S. macrophyllus* flowers permits nectar and pollen resources to be available to a broad array of insect visitors. From October to November 2006, a wide range of native and non-native insect species was observed foraging for nectar at flowers. Honey bees were by far the most frequent visitor to *S. macrophyllus* flowers. Feral honey bees have spread over much of Hawai`i since their introduction in 1857 (Roddy and Tsutsumi 1997) collecting both pollen and nectar from a wide variety of native and non-native plant species (Arita *et al.* 1989). It is uncertain the impact honey bees may be having on the fitness of native plants. As the dominant visitor of *S. macrophyllus* flowers, it is possible honey bees may be deterring native pollinators and/or depleting nectar resources shared with native pollinators.

Other common floral visitors observed on *S. macrophyllus* were the seed bug *Nysius* sp, the golden paper wasp *Polistes aurifer*, and thrips (Phlaeothripidae of unknown species). Seed bugs primarily feed on seeds, but adults may also feed on the nectar of flowers (Beardsley 1977). On *S. macrophyllus*, the native seed bugs were observed aggregating on inflorescences and sucking up nectar collected at the base of the corolla. Paper wasps are regarded as pollinators of plants, but are far less efficient at pollinating flowers than honey bees. Unlike honey bees, paper wasps have little hair on their bodies for pollen to adhere and be moved from one flower to another. To meet their energy requirements, adult paper wasps visit flowers to obtain nectar, but also capture insect prey to provide their larvae with a necessary protein diet (Hunt 2007). Many species of thrips are abundant in the flowers of a wide range of plant taxa feeding on pollen and nectar, but are regarded as minor pollinators (Kirk 1997). Further

research may be needed to determine whether any of these floral visitors play a role in pollinating the flowers of *S. macrophyllus*.

The abundance of floral visitors corresponded with the prolific flowering (Fig. 36 and 37) and increased vegetative growth during fall 2006. In the following year, significant die-back was observed and availability of flowers was less pronounced in the fall. From casual observations, visitor abundance also decreased at this time, and it is likely that the relatively low flower abundance was not sufficient to attract pollinators that are drawn to mass flowering such as honey bees (Sih and Baltus 1987, Agren 1996).

The hand-pollination experiments revealed no significant increase in fruit set compared to open pollination, suggesting *S. macrophyllus* was not pollen-limited in the fall of 2006. Overall, fruit set was low which may indicate reproductive success in *S. macrophyllus* is limited by the amount of resources allocated for seed production (Stephenson 1981, Sutherland 1986). However, fruit set in both open- and hand-pollinated inflorescences during the experiment was higher than that detected in inflorescences tagged to determine success of fruit production. It is likely that fruit set is highly variable among plants, seasons, and years.

Success of Fruit Production

Of the five species studied, only *M. hawaiiensis* and *Z. dipetalum* showed relatively high proportions of successful transition from flower to fruit, indicating adequate levels of pollination and fertilization. *Sicyos macrophyllus* did not have a high proportion of successful fruit production based on the number of female flowers that became fruit, but the large numbers of flowers of both sexes led to a heavy fruit crop in both monitoring years. All three of these plant species have breeding systems that likely promote out-crossing. *Zanthoxylum dipetalum* is the only dioecious species studied, so it is essentially an obligate out-crosser although we detected very low levels of fruit on otherwise male trees. This imperfection of dioecism is apparently not rare (Baker 1967). Both species of *Melicope* and the vine *S. macrophyllus* are monoecious with unisexual flowers of both genders on the same plant. Dioecism and mixed breeding systems are more prevalent in Hawai'i than in comparable continental systems (Carlquist 1980); although the incidence of dioecy is not as high as previously estimated, it remains the highest known in the world (Sakai *et al.* 1995).

Zanthoxylum dipetalum had the highest success rate of fruit production from tagged flowers with 32% of buds/flowers producing small green fruit and 16% persisting to become large green fruit that were the same size as mature fruit. The more common of the two *Melicope* studied, *M. hawaiiensis*, also had a high percentage of buds/flowers that formed immature fruit (18%), although the detected transition rate to mature fruit was low. The observed rate of mature fruit production was probably artificially low, because capsules that turned brown and dispersed between monitoring periods were not counted as successful transitions to mature fruit.

The 5% success rate of *S. macrophyllus* buds/flowers to large green fruit is lower than those of the two successful fruit-forming species, but the abundance of small female flowers grouped together in globose inflorescences led to large crops of both mature and immature fruit at monitored plants. There is variability in the level of successful fruit production detected, because the open-pollinated inflorescences sampled in the pollination experiment produced a much higher percentage of fruit (14.6%) than did the large sample of tagged inflorescences monitored for fruit formation. The monthly interval in the tagged inflorescence monitoring may not have been short enough to detect fast-developing fruit before they dispersed.

The fruit production of the two endangered species studied (*H. giffardianus* and *M. zahlbruckneri*) was very low, by contrast with the three moderately successful fruit-producing species. The successful transition of flower to fruit capsule was only 1.8% for *H. giffardianus*, a species with large perfect flowers morphologically suited to cross-pollination with a stigma exerted far above anthers of the short staminal column. The fruit production detected in *M. zahlbruckneri* was the lowest of the five species studied; the transition rate of buds/flowers to immature fruit was only 0.5%, and the production of mature capsules was a miniscule 0.2%. Limited fruit production appears to be part of the problem for this species, and both lack of pollination and insect predation of young fruit (see below) are implicated as important limiting factors for *M. zahlbruckneri*. Fruit set amounting to 1% or less has been considered a very low level of productivity in other studies of various plant species (Anderson and Hill 2002, Armstrong and Irvine 1989, Bertin 1982, Zimmerman and Aide 1989). Such a low fruit set is generally interpreted as a failure of effective pollination, although lack of dispersal and characteristics of breeding systems may also contribute to low productivity.

Comparable information on productivity of other rare plant species in Hawai`i is largely lacking. While research on pollination ecology, seed germination, and seedling establishment is recommended in the recovery plans for endangered plants, no information on the reproductive potential of the endangered plants is presented in species accounts (U. S. Fish and Wildlife 1996, 1997a). Among Hawaiian plants, lobelioids, both common and rare species, have generated considerable interest in pollination biology (Cory 1984, Palmer 1996, Drake and Morden 2006). Because most *Clermontia* and *Cyanea* are capable of self-fertilization, fruit formation and seed production is generally good, even in rare species. Some work has been done on fruit set in common Hawaiian plants, particularly community dominants. Carpenter (1976) found high percentages of fruit set in *Metrosideros polymorpha* (then known as *M. collina*); this species may self-pollinate, but fruit set is improved by the pollinating activities of insects and birds. When red-flowered trees were pollinated by both insects and native birds, fruit set was >55%. Reported fruit set was somewhat lower in *Acacia koa*, the dominant tree on Mauna Loa, where insect-pollinated flowers achieved 15% fruit set and the proportion of self-pollinated flowers that set fruit was 2.7% (Lanner 1965). Van Riper (1980) estimated the productivity of the subalpine forest dominant *Sophora chrysophylla* as one pod for every six flowers (17%).

Seed Germination and Soil Seed Banks

Seed germination rates were high in *Z. dipetalum* var. *dipetalum*, moderate in *H. giffardianus* and *S. macrophyllus*, low in *M. hawaiiensis*, and almost non-existent in *M. zahlbruckneri*. Greenhouse trials with fresh seeds of *Z. dipetalum* had mean germination rates of 55-56%, and individual flats had seed germination rates as high as 67%. No special treatment was given to seeds, so even higher rates might be possible with pre-treatment, soaking, or scarification. There was little difference in germination rates of seeds sown during three seasons (winter, summer, or fall). Time to first germination was almost two months, a month longer than that reported for the genus by Lilleeng-Rosenberger (2005), who was working at a low-elevation greenhouse. While most germination occurred within a few months, seedlings continued to appear for 10 months. Viability of the seeds also tested positive or strongly positive. Recent propagation efforts at HAVO also found a high germination rate for this species, with 100% germination reported for the best trial (McDaniel *et al.* unpublished). Past propagation efforts in the Park were less successful with reported germination rates of 0 to

15% (Zimmer unpublished). Stratton *et al* (1998) reported a germination rate of 20-50% for this species.

Hibiscadelphus giffardianus germination rates were quite variable, and the 26-31% germination rates achieved in the most successful trials were relatively low, compared with the most successful germination rate of 75% reported by HAVO propagation specialists (McDaniel *et al.* unpublished). Zimmer (unpublished) reported 67% as the best germination rate for this species, but these seeds may have represented the hybrid *Hibiscadelphus x puakuahiwi*. In our study, seed viability did not appear to be a factor in reducing germination rates because seeds tested positive and viable. The relatively rapid first appearance and continued germination for several months that we observed were also reported by Lilleeng-Rosenberger (2005). She suggested soaking seeds of this species for two hours in warm water to increase germination, so rates of germination in the current study may be less than maximum for this species. Greenhouse germination rates in the current study were much higher than field germination rates at Kīpuka Kī. Despite the very low rates of seedling establishment from field-sowed seeds (2-10%), this test demonstrated that recruitment from planted trees was possible if seeds were moved slightly away from parent trees to sites without a cover of alien grass. The natural leaf litter at Kīpuka Kī may have been beneficial to the field-sowed seeds by hiding them from foraging rats and potentially maintaining moist conditions for the relatively large *Hibiscadelphus* seeds. McAlpine and Drake (2002) found that a litter layer increased germination of the large-seeded *Alectryon excelsus* in New Zealand, which they attributed to reduction of seed desiccation. Moving the seeds away from the parent plants may also have reduced the likelihood of rat predation in the unprotected plots (Chimera 2004).

The difference in the germination rates of *Sicyos macrophyllus* using untreated versus peeled seeds is evidence that the thick inner fruit wall is contributing to physical dormancy of the seeds. Our results were improved by either peeling the seeds out of the husk or clipping the end of the dried inner fruit wall containing the seed. With either of these treatments, germination began within two to eight weeks. The germination rates of 27-42% we achieved with nicking or peeling might be increased by soaking seeds in water as suggested for the genus by Lilleeng-Rosenberger (2005). Our tests of seed viability indicated that most were viable, and this was not limiting germination of *S. macrophyllus*. Baskin *et al.* (2004) reported that seeds of the genus *Sicyos* have combinational dormancy with both an impermeable fruit

coat and physiological dormancy of the embryo. Physiological dormancy usually requires a time period of embryo maturation before the seed or fruit coat becomes permeable to water. This combinational dormancy likely prevents seeds of dry forest species from germinating after brief rains during dry seasons (Baskin and Baskin 1998).

Melicope hawaiiensis had relatively low germination rates in the greenhouse trials, which used fresh seeds. Most tested seeds were positive in the viability trial, although for 30% of seeds the response was only weakly positive. Seedlings in 2006 and 2007 did not appear for six months. This delayed time to germination for *Melicope* seeds was also reported by Lilleeng-Rosenberger (2005), who found that some Kaua'i species required eight months in the greenhouse before seedlings appeared. Previous work with the species at HAVO indicated time to germination varied from two months to one year (Joy Hosokawa pers. comm.). Propagation work with this species 30 years ago at HAVO indicated a best germination rate of 26% and 40 days to germination (Zimmer unpublished). The seeds collected from the soil seed bank cores in the current study germinated relatively quickly (within two months) and achieved a germination rate as high as 44%, four times higher than the mean of the best trial with fresh seeds. Another Hawaiian species in this genus is reported to have physiological dormancy (Baskin *et al.* 2004); time spent in the soil may have allowed the embryos of *M. hawaiiensis* seeds to mature so that the dormancy was broken and seeds were able to germinate more quickly after planting.

By contrast with the low rates of germination achieved with *M. hawaiiensis*, only one seed germinated in regular tests of the endangered *M. zahlbruckneri*. This lack of germination cannot be ascribed to low seed viability since all seeds tested positive. When cutting sample seeds for the viability test, we noted that the seed coat was much thicker than in the more common species, measuring three times as thick as that of *M. hawaiiensis*. This may indicate an even more pronounced physical dormancy in this species than in other members of the genus. Previous attempts to propagate this rare species at HAVO have also been unsuccessful; Zimmer (unpublished) had little success with seeds, cuttings, or air-layers and only successfully planted one seedling. Belfield *et al.* (unpublished) had limited success with cuttings and germinated only one seed.

Soil seed bank results indicated that among the five species studied, only *M. hawaiiensis* and *S. macrophyllus* had a sizable seed bank. The few intact seeds detected in the soil beneath

Z. dipetalum trees were at very low density and were probably transient; large seeds with rough seed coats do not typically persist in soil seed banks (Baskin and Baskin 1998). The largest soil seed bank detected was that of *M. hawaiiensis*; the later germination of seeds in the greenhouse indicated that more than a third of intact seeds of one tree were viable and capable of recruiting seedlings. The soil bank held seeds in three seasons, but we did not gather enough data in consecutive seasons to demonstrate whether the seed bank was persistent or transient (Garwood 1989). Our detection of rat-predated seeds in the soil bank of both species of *Melicope* indicated that predation may be a problem for these mesic forest trees. Rats, rather than mice, (*Mus musculus*) are likely the seed predator here based on the size of the tooth marks on seed remnants (Wilmshurst and Higham 2004). Drake (1998) did not detect seeds of the related *M. radiata* (alani) in the soil of a HAVO rain forest at Kealakomo despite the presence of the tree in the surrounding vegetation, and he did not observe rat predation of *Melicope* seeds in his study (Drake 1993).

The second largest soil seed bank that we detected, that of *S. macrophyllus*, also provided evidence of rat predation with more than half of recovered fruit showing signs of rat gnawing and seed consumption. The summer seed bank was far greater than that of the fall season, indicating that the seeds in the soil are probably transient rather than persistent. Predation by rodents appeared to be proportionally similar in the winter and summer.

No soil seed bank was detected for *H. giffardianus* in two seasons of sampling. Only one seed, which did not germinate, and rat-predated fragments were recovered from the soil. It is unclear whether predated fragments represented seeds incorporated into the soil or the remains of seeds predated from capsules still on the tree. Similarly, the soil samples beneath fruiting individuals of *M. zahlbruckneri* provided no whole seeds, and with one exception only fragments predated by rats were recovered. For this species, the seeds were likely predated on the ground; in more than two years of sampling we observed only one instance of a potentially rat-damaged capsule on a *M. zahlbruckneri* tree.

Two recent studies of the soil seed banks within rain forests of HAVO reported relatively low numbers of large seeds of native tree and shrub species. In his study of the seed rain and seed bank of a forest at Kealakomo, Drake (1998) found high numbers of the small seeds of the dominant *Metrosideros polymorpha* and the shrubs *Pipturus albidus* (māmaki) and *Dodonaea viscosa* (‘a`ali`i), but extremely low numbers of the large seeds of *Myrsine* spp. (kōlea),

Coprosma menziesii (pilo), and *Leptecophylla tameiameia* (pūkiawe). Seeds of other native trees found in the forest were completely absent from the soil seed bank. A similar paucity of native tree seeds was reported by Loh and Daehler (2008) in their study of the seed bank of a forest on Kīlauea Crater rim. In both cases, the researchers concluded that the composition of the forest soil seed bank varied greatly from the seed rain and the species composition of the current vegetation. Seeds of alien plants dominated the soil seed bank, creating a vulnerability to invasion following disturbance to the native forest. Short survival time of seeds in soil is typical of most species of tropical rainforests, where “prompt germination” is the most common strategy (Vázquez-Yanes and Orozco-Segovia 1993).

Impacts of Rodents

Rodents, most likely rats (*Rattus* spp.) based on the size of tooth marks, were confirmed as seed predators of four of the five species studied in Kīpuka Puauulu and Kīpuka Kī. *Zanthoxylum dipetalum* was the only species in the group whose seeds or fruit were not taken by rodents when offered and were not typically damaged while on the tree. However, even this species displayed severe bark-stripping on three of 53 trees visited regularly for more than two years. This low level of stripping is perhaps a chance event involving few animals. Previous observations of rat bark-stripping on planted saplings led us to enclose a sample of recent plantings in rodent-proof cylinders, but no damage was observed in unprotected control saplings. Based on the current study, rats cannot be considered a significant limiting factor for this species.

Rats have been observed to strip bark of several trees of common and rare species in Kīpuka Puauulu (Russell 1980, Stone 1985) and other Hawaiian forests. Scowcroft and Sakai (1984) reported that more than half of the young *Acacia koa* trees in silviculture stands were bark-stripped by rats at three sites on Hawai`i and Maui. Duffy and Gardner (1993) observed rat bark stripping on 75% of the *Sophora chrysophylla* they studied, and a few young trees were killed by this depredation.

Hibiscadelphus giffardianus displayed the most severe and repeated rat damage of all the species observed in the mesic forests. Seeds on the ground were readily taken and consumed by rats, and a low but persistent level of seed predation was detected in ripe capsules still attached to trees. Flower and bud predation and severe bark stripping were

episodic events observed repeatedly during the study. The complete lack of natural seedling recruitment was likely due in part to seed predation, because fruit capsules were produced at a low level by most trees, seed germination was possible even under field conditions, and alien grass cover had been removed from the planting sites of most trees. Although self-incompatibility and inefficient pollination were also indicated for this species, predation by rats appeared to be contributing to its lack of recovery within its restored habitat.

Past studies have also documented damage to *H. giffardianus* attributed to rats. Baker and Allen (1978 and unpublished) reported severe bark stripping to the species and hybrids in HAVO. They noted that major limbs of trees were killed by this activity, which was most acute during the dry summer. We also observed severe bark-stripping, but the episodes occurred in the spring. None of the monitored trees died during our study, but at least one non-sampled tree in the study area was completely girdled by rats and died. Baker and Allen were reporting on a small sample of large trees approximately 20 years old; we included the remaining original trees in our study, but most of our monitored trees were less than 10 years old. Severity of bark-stripping damage may be proportional to the size and number of branches on *Hibiscadelphus* trees and may be related to the proximity of other tree species.

All bark-stripped *H. giffardianus* trees in our study were adjacent to and in contact with *Pipturus albidus*, while none of the trees without damage had *Pipturus* as a near neighbor. In a previous study carried out in Kīpuka Puʻaʻulu, 80% of *P. albidus* trees had signs of rat movement on tracking boards attached to trunks, representing the highest percentage of more than 10 tree species studied (Forbes and Stone unpublished).

Damage to reproductive structures and seed predation by rats may be more detrimental to the continued existence of *H. giffardianus* plantings than bark-stripping. Baker and Allen (1976) reported that up to 75% of flowers were damaged by rat feeding, and 88% of the seed crop of trees they studied was consumed by rats (Baker and Allen 1978). While we only detected rat damage on 10% of the fruit attached to our sample trees, we do not know the fate of the seeds once they dropped to the ground. The evidence from our seed offering in which all unprotected seeds in Kīpuka Puʻaʻulu were quickly taken supports the contention of Baker and Allen of destruction of most of the seeds of *Hibiscadelphus* by rats. During this study we did not observe the feeding or husking stations reported by Baker and Allen (1978), although rodents left husked remnants beside seed offering trays.

Rat predation appeared to be severe, although episodic, on *M. hawaiiensis* seeds, both while in the capsules on trees and after dropping to the ground and incorporation into the soil seed bank. At the beginning of the study a large cache of predated capsules was observed beneath one tree (Thomas Belfield pers. comm). Such rat husking stations beneath trees have been reported elsewhere in the Pacific (McConkey *et al.* 2003). By contrast, the endangered *M. zahlbruckneri* only once displayed a rat-damaged capsules attached to a tree, but rat predation was detected on remnant seeds within the soil. For both species of *Melicope*, insect seed predation seemed to be more severe and persistent than did rat damage, but both predators may contribute to preventing seedling recruitment in these rare trees. *Melicope zahlbruckneri* had an additional problem forming fruit, implicating failure of pollination. The recovery plan for *M. zahlbruckneri* (U. S. Fish and Wildlife Service 1997a) suggested both rodent control and control of insect damage as needed recovery actions.

Sicyos macrophyllus seed predation was never observed while fruit clusters were attached to the vines, but gnawing was noted on a high percentage of fruit fragments recovered from the soil seed bank, and rodents also consumed or removed offered seeds at multiple sites. As with *Melicope*, size of tooth marks implicated rats rather than mice. Loss of offered fruit in closed box controls may have been due to withering of fruit that formed without producing seeds. We noted that 12% of full-size fruit collected for the final seed germination trial were empty inside when peeled and clipped. The lack of on-plant fruit predation may be explained by the fact that fruit are borne on slender non-woody stems of the vine that would likely not support the weight of a rat. During more than two years of monitoring, we noted rat damage to the fleshy stems of *Sicyos* only once. When the fruit fall, rats may be attracted to the flesh surrounding the seed; the seeds of other dry and mesic forest species are more vulnerable to predation by rats when their fruit flesh is intact and when they are near the parent plant (Chimera 2004).

We did not carry out rat trapping as part of the current study, and the identity of the depredating rat is not known with certainty. However, the rodent species composition of both Kīpuka Puaulu and Kīpuka Kī was dominated by black rats (*Rattus rattus*) in previous studies (Spurr *et al.* 2002, Forbes and Stone unpublished, Tomich 1981). Prevalence of black rats at middle- to high-elevation forests is typical in Hawai`i (Stone 1985, Tomich 1986). Relative rat densities appear to be similar in the two kīpuka and are high compared with other HAVO

forests. While we do not have absolute densities of rats in the mesic forest study area, Spurr *et al.* (2002) reported 15.4 black rats per 100 trap nights in Kīpuka Puauulu and 20 black rats per 100 nights in Kīpuka Kī. Rats per 100 trap nights reported by Forbes and Stone (unpublished) were higher in Kīpuka Puauulu than in five other forest sites in HAVO. Black rat numbers reported for the two kīpuka by Spurr *et al.* are similar to those observed at Waikamoi wet forest on Maui (Sugihara 1997) and Hakalau Forest Refuge on Mauna Kea (Lindsey *et al.* (1999) but double the numbers reported at Hanawī, Maui (Sugihara 1997).

Rats are well known as seed predators in Hawai`i and elsewhere in the world. Sugihara (1997) determined that 60% of the rats in two Maui rainforests consumed native fruit and seeds; he identified species of *Rubus*, *Pittosporum*, and *Coprosma* in the stomach contents of rats he examined. Cole *et al.* (2000) determined that native species made up most of the plant material consumed by rats in native shrubland of Haleakalā on Maui; fruit and seeds were important components of the rat diet. On Mauna Kea, a small sample of black rats consumed fruit and seeds of native trees, as well as alien grass seeds (Amarasekare 1994). Cabin *et al.* (2000) implicated rats as seed predators of the fleshy-fruited *Diospyros sandwicensis* (lama) in dry forest of leeward Hawai`i, where seedling recruitment of this community dominant was seen only after rodent poisoning.

Few studies in Hawai`i have been carried out on the impacts of rats on rare plant species, although systematic assessments of rat food preferences and seed predation of native species are currently underway (Aaron Shiels pers. comm.). Studies of rat feeding and simulated rat damage to large-seeded *Pritchardia* palm (loulou) indicated that rats severely damaged most seeds, but those damaged up to 45% of their mass could survive and germinate (Pérez *et al.* 2008). Rats have also been observed to be seed predators of *Pritchardia* spp. in the palms' natural habitat (Male and Loeffler 1997). Rat seed predation has been observed on a number of rare and uncommon tree species on Maui (Medeiros *et al.* 1986, Chimera 2004).

A recent study of the impacts of rats on islands of French Polynesia reported that 15 threatened plant species were damaged by rat seed predation, primarily large-seeded species (Meyer and Butaud 2008). Rat seed predation and reduction of seedling recruitment has been noted in many studies of other Pacific islands (McConkey and Drake 2002, Moles and Drake 1999, Campbell and Atkinson 2002, Allen *et al.* 1994). When toxicants are approved for large-

scale treatment of forests in Hawai`i, subsequent studies may reveal the scope of the impact of alien rodents, not only on the recruitment of rare plant species, but also on the basic species composition and structure of Hawaiian forests.

Seedling Predation, Potential Seed Dispersal, and Flower Nectar-robbing by Birds

Birds were implicated as seedling predators for one monitored species (*Z. dipetalum*) and as nectar-robbers for a second species (*H. giffardianus*). Kalij pheasant damage was observed on young seedlings of *Z. dipetalum*, including both natural seedlings beneath parent trees and seedlings germinated from planted seeds. While the bite marks of the birds are obvious when fresh, once most of the leaf is gone from either repeated feeding by pheasants or severe insect feeding, it is not clear what causes the loss of the seedlings. The results of enclosure experiments with seeds were not completely definitive, although length of survival of a small number of seedlings was improved by installation of pheasant-proof poultry netting compared with unprotected control plots in one test. The experiment with natural seedlings beneath four parent trees displayed statistically greater mortality of seedlings outside enclosures than within, but the ultimate cause of death of seedlings may be due to several factors. Damage from leaf-feeding insects (of unknown species) affected a great number of natural seedlings, and non-native slugs were present. The appearance of *Z. dipetalum* seedlings is not a rare occurrence; two of the trees with natural seedlings in 2008 have been observed with seedlings in previous years, but seedlings did not survive (Thomas Belfield pers. comm.). Kalij pheasants have been in HAVO since 1977 (Lewin and Lewin 1984), so their impacts on the structure of both common and rare native plant populations may not yet have been fully manifested in slow-growing tree species.

Kalij pheasants were previously identified as seedling predators in a rare plant restoration project within Kīpuka Puauulu and Kīpuka Kī. Belfield *et al.* (unpublished) observed Kalij pheasant damage and feeding on nine of ten tree and shrub species planted in the mesic forests. *Kadua affinis* (manono) and *Urera glabra* (ōpuhe) were particularly badly damaged, but all planted species except *Charpentiera obovata* (pāpala) showed signs of leaf feeding by Kalij, and several seedlings were uprooted by digging at the base of plants. Pheasant damage was greatest on small seedlings, and the birds' ability to disrupt plant growth dissipated as plants increased in height and foliage grew out of reach.

Recent research on food habits of Kalij pheasants identified a large number of both native and alien fruit taken by the pheasants, which destroyed most of the seeds they consumed (Postelli *et al.* 2006). We found no evidence that Kalij pheasants fed on the seeds of two target species, based on the seed offerings that were made outside exclosures or bait stations for *Z. dipetalum* and *H. giffardianus*. Seed offerings of two other species were inaccessible to pheasants. Previous work on food habits of Kalij pheasants, based on analyses of crops and gizzards, identified more than 19 plants consumed, including fruit, seeds, and leaf material (Lewin and Lewin 1984).

Postelli *et al.* (2006) concluded that Kalij pheasants were acting as seed dispersers for many of the native and non-native plants they studied, particularly those with small seed size. The large number of fruit and seeds consumed led to dispersal even though most seeds were destroyed by ingestion. Seed dispersal may well be a limiting factor for some of the five species we studied. Nothing is known to disperse *H. giffardianus*, leading to the dropping of fruit on the ground and the likely consumption of seeds by alien rats. By contrast, *Melicope* seeds have been identified as part of the diet of `Ōma`o or Hawaiian Thrush (*Myadestes obscurus*) (Wakelee and Fancy 1999) and the Hawaiian Crow or `Alalā (*Corvus hawaiiensis*) (Banko *et al.* 2002). The brown seeds of other species of *Melicope* are known to be high in protein content (Sakai and Carpenter 1990). *Melicope hawaiiensis*, at least, has a relatively high number of large trees that produce copious fruit, and it is highly likely that resident `Ōma`o have encountered this potential food item in their territories. Seed dispersal by `Ōma`o may have led to the recruitment of young *M. hawaiiensis* plants we noted in Kīpuka Puauulu; all young trees we encountered were well removed from fruiting adults.

The `Alalā or Hawaiian Crow, formerly present in the mesic forests of Mauna Loa (Banko and Banko 1980), may have been the dispersal agent for the large, shiny black seeds of *Z. dipetalum*, a species related to *Melicope*. `Alalā have been observed feeding on fruit similar in size to the seeds of *Z. dipetalum* (Sakai *et al.* 1986). Certainly, the presentation of the shiny seeds dangling from opened ripe fruit capsules is suggestive of an adaptation to bird dispersal. We have no speculation as to the potential dispersal agent of *Sicyos macrophyllus*. A suite of alien birds has been identified as important dispersers of native forest trees and shrubs on Maui (Foster and Robinson 2007). While most of the alien frugivorous birds studied on Maui are also

present within Kīpuka Puaulu, their feeding habits and seed dispersal activities are unstudied within the Park.

Nectar-robbing was noted on *Hibiscadelphus giffardianus* flowers during observations of potential pollinators. Both native `Amakihi and alien Japanese White-eyes were observed visiting flowers and feeding on nectar through perforations at the base of the large tubular flowers; this activity was also reported at *Hibiscadelphus* flowers in the two kīpuka more than 30 years ago (Yutzy and Yutzy unpublished and Baker and Allen unpublished). Kaua`i `Amakihi, *Hemignathus kauaiensis*, have also been observed robbing nectar from flowers of native tree species, such as *Clermontia fauriei* on Kaua`i (Drake and Morden 2006). When we quantified the number of flowers nectar-robbled in Kīpuka Puaulu and Kīpuka Kī, we found that most flowers showed perforations. Kīpuka Kī had higher rates of nectar-robbing, perhaps because there are fewer food plants available in the more open understory of this kīpuka as compared to Kīpuka Puaulu. `Amakihi are certainly more obvious in Kī, but we do not have current data to support a difference in populations in the two kīpuka. *Hibiscadelphus* nectar appeared to be a more important food source for birds in the winter and spring than in the summer, corresponding to a paucity of *Metrosideros* flowers during a low period in flower production of that species at middle elevations on Mauna Loa (Porter 1973, Carpenter 1976). The amount of nectar produced by an individual *Hibiscadelphus* flower has been reported to be 5 cc (Baker and Allen 1976b), a much greater amount than could be expected from a single *Metrosideros* or *Sophora* flower.

Insect Seed Predation and Seedling Herbivory

Insects were recognized as seed predators of both species of *Melicope* in the current study, but no indication of extensive insect damage was noted for seeds of the other three species studied. The fruit and seeds of *Zanthoxylum dipetalum*, a member of the same family as *Melicope*, were never observed with obvious insect damage while on the trees. Seed predation was observed on approximately one quarter of collected capsules of *M. hawaiiensis*, but predation was far more severe on the endangered *M. zahlbruckneri*, where more than two-thirds of capsules showed signs of insect damage in each collecting period, and predation ranged as high as 95-100%. There was also a difference in the number of intact seeds remaining in predated capsules of the two species. Typically, only one seed remained in large predated *M. zahlbruckneri* capsules, but as many as three intact seeds were noted in those of

predated *M. hawaiiensis* fruit. Intact seeds were found in carpels unoccupied by insect larvae. The two species have very similar fruit forms, and the maximum number of seeds of either species is eight when all four carpels develop. The developing larvae of the *Prays* moth consume seeds of *Melicope* (Swezy 1954) and leave behind frass. We found evidence of larvae in developing capsules of *M. zahlbruckneri* as small as 8 mm, which is approximately one-third mature fruit size. Larvae pupate within the capsule, and when adults emerge, leave the capsule by means of a round exit hole. We never saw any intact seeds within a carpel inhabited by a *Prays* larva. Multiple carpels of a single fruit could be host to multiple moth larvae, and we saw evidence of larvae moving between carpels because some fruit capsules had holes in the internal septa.

The species of *Prays* responsible for predation of *Melicope* seeds appear to be undescribed and endemic. This genus was formerly thought to contain just one introduced and one native species in Hawai`i, but it is now recognized that several single-island endemic species are present on at least six of the Hawaiian Islands (Zimmerman 1978). Only *P. fulvocanella* is currently described (Nishida 2002); this species was formerly considered to inhabit all the islands and was described as a predator of the buds and seeds of many *Melicope* (*Pelea*) species (Swezy 1954). Seed predation by native *Prays* has been recognized as a reason for decline and a current threat to four endangered species of *Melicope* on Maui (U. S. Fish and Wildlife Service 1997b). It is not known whether the species of *Prays* impacting *M. zahlbruckneri* are rare or common native insects. Nonetheless, if seeds of the endangered *Melicope* are to be available for natural reproduction or future propagation efforts, it may be desirable to control insect damage to fruit.

Future research is warranted to study the native insects in more detail and establish their host-specificity. Other topics of potential research might include the effects on *Melicope* and dependent insects of systemic insecticides and the efficacy of exclusionary devices for inflorescences following pollination. However, the practicality of treating insect pests on large trees in natural forests is questionable, and there is some risk to the ecosystem if native insects are reduced. Other propagation techniques involving air-layering and cuttings are being considered for use with *M. zahlbruckneri* (Keali`i Bio pers. comm.); if successful vegetative propagation techniques are developed, Park managers might be able to increase numbers of

reproducing plants and establish new populations of this critically endangered tree within HAVO, even without successful seed germination.

While tagging *H. giffardianus* flowers to determine level of fruit production, we noted sap beetles inside flower corollas. Beetles were particularly abundant in senescent and fallen flowers. Subsequently, beetles within *Hibiscadelphus* flowers were identified as *Prosopaeus subaeneus*, an endemic species in the Nitidulidae that is common in flowers of native trees and shrubs of wet and mesic forests (Ewing 2007). The beetles feed on nectar, fungi, yeasts, and pollen (Lachance *et al.* 2003), and are not thought to be damaging to the flowers themselves.

Foliar damage to seedlings due to unknown insects was noted for both *H. giffardianus* and *Z. dipetalum*. We had no small seedlings of either *Melicope* species to observe, and no conspicuous insect damage was noted on planted seedlings of *S. macrophyllus*. Most *Hibiscadelphus* seedlings that established within seeding plots were attacked by insects when they were small. Insects were not collected and identified, but based on the type of damage observed, herbivores almost certainly included Fuller rose beetle (*Asynonychus godmani*) and species of lepidopteran caterpillars. Typically insect damage to leaves decreased when seedlings of this species achieved greater height. Foliar damage to *Z. dipetalum* seedlings was frequently severe, and most natural seedlings and seedlings germinated from seed plots showed at least light foliar feeding. The insects responsible for the observed damage were never seen on the plants during the day and were not collected. Zimmer (unpublished) and current HAVO greenhouse staff (Susan Dale pers. comm.) reported foliar damage to greenhouse-grown *Z. dipetalum* seedlings by caterpillars of the citrus swallowtail butterfly (*Papilio xuthus*).

Alien Grasses

Two of the target rare plant species (*S. macrophyllus*, and *Z. dipetalum*) had either direct seeding experiments or planted seedlings in areas with and without alien grass. Grass impacts on the two *Melicope* species were not studied because of the paucity of seeds and the very low percentage germination observed in the greenhouse. *Hibiscadelphus giffardianus* seeding was only carried out in grass-free sites.

For *S. macrophyllus*, no seed germination was observed in plots with a heavy cover of alien grass. Adjacent seed plots without grass did exhibit seed germination and establishment,

of one seedling. The alien grass species covering the *S. macrophyllus* seed plots in Kīpuka Kī was *Ehrharta stipoides*. This is the most common alien grass in Kīpuka Kī and has been the target of alien plant control efforts since the early 1990s (Tunison and Stone 1992). It is also common beneath tree canopy in Kīpuka Puau. A perennial bunch grass with stems to 0.75 m, *E. stipoides* is a prolific seeder, which seasonally produces abundant fruit with long barbed awns that aid in dispersal (Whitney *et al.* 1964).

While we did not test seed germination of *Z. dipetalum* in grass and no-grass plots, we did plant small seedlings from greenhouse germination trials in a sunny grassy site and an adjacent shady site beneath native trees. The dominant cover in the sunny clearing was *Cynodon dactylon*, Bermuda grass, an alien species introduced as a pasture grass at low elevations (Whitney *et al.* 1964). This grass is probably a relict from the era of ranching in the Mauna Loa Strip, and it remains within the kīpuka despite alien plant control efforts. The grassy site was clearly inhospitable to the seedlings, which did not survive more than two months. These losses may have been due to competition for resources with the alien grass, or the seedlings may not have been able to survive the full sunlight of the site. Seedlings exhibited dry leaves and showed signs of water stress. All natural seedlings observed in the current study and past reports (Morris unpublished) have been in shade beneath parent trees.

D'Antonio *et al.* (1998) found improved growth of native shrubs in a dry *Metrosideros* woodland within HAVO after alien grasses were removed. They also recorded an increase in native shrub seedling recruitment in grass removal plots, which they attributed to increased light and temperature. Dense grass cover or grass litter have been shown to limit the establishment of woody plants in many studies (D'Antonio and Vitousek 1992). Grass competition may reduce water, light, or nutrients and restrict growth or establishment of other species. Since water use, nutrient levels, or light were not measured in the current study, we have no data on the mode of suppression that grasses have on native seedlings.

CONCLUSIONS

Limiting Factors of Five Species

Hibiscadelphus giffardianus. Rat predation of seeds and bark-stripping were identified as limiting factors, but low fruit production likely resulting from the inter-relatedness of the planted population and the low availability of mates in a self-incompatible species may present the most serious limitations to reproduction and recovery. Loss of original pollinators was also recognized as a possible problem.

Melicope hawaiiensis. Rat and native insect predation of seeds limited the amount of potential propagation material available in the habitat of this species. Natural seedling recruitment was very low. Pollination was clearly occurring although the agent was identified as an alien insect species, and fruit production appeared to be at an acceptable level.

Melicope zahlbruckneri. Native insect predation greatly reduced the number of seeds produced by the most productive trees. Rats depredated seeds that fell to the ground and may have prevented development of a soil seed bank. The very low proportion of flowers that became fruit indicated a lack of successful pollination or self-incompatibility.

Sicyos macrophyllus. Rat predation of seeds on the ground reduced the number of seeds available for natural germination. The role of alien grasses in limiting natural seedling recruitment was not confirmed but is suspected. Most floral visitors were alien insect species, but pollen supplementation did not improve fruit set, indicating that pollination is not limited.

Zanthoxylum dipetalum var. *dipetalum*. Seedling recruitment appeared to be the most vulnerable life stage for this species. Both alien Kalij pheasants and unknown insect species were implicated as seedling predators/herbivores.

Suggestions for Future Work

Hibiscadelphus giffardianus. Investigation into the ability of alien Japanese White-eyes to effectively pollinate visited flowers would supplement our findings. Additional research examining pollen tube growth in styles of *H. giffardianus* hand-pollinated with pollen from the closely related *H. hualalaiensis* may be useful to confirm self-incompatibility for this species. Deliberate hybridization with the closely related *H. hualalaiensis* and establishment of a hybrid

population distant from that of *H. giffardianus* might answer the question of whether such hybrids could be self-sustaining, although production of hybrids through hand-pollination may be difficult. Use of hybrids to backcross with “pure” *H. giffardianus* at a site distant from current trees might achieve a reproducing population with the characteristics of the native species, although this would be a long-term project. Development of techniques to temporarily reduce rat populations during periods of peak fruit production in sections of the kīpuka supporting the rare tree might allow a low level of seedling recruitment.

Melicope hawaiiensis and *M. zahlbruckneri*. Further study of the native insect seed predators may provide insight into their relationship with native *Melicope* species. Research into systemic insecticides to reduce loss of seeds to native insects may be warranted, but should take into consideration impacts on native insects. Investigation into ways to break seed dormancy and increase greenhouse seed germination would potentially allow future propagation and planting of the two species in HAVO. In the short-term, success with vegetative propagation techniques might lead to increased numbers of individuals. Conservation of *Melicope zahlbruckneri* might benefit from identification of potential pollinators of the endangered species. Seed dispersal studies might establish whether this is a significant limiting factor with either species.

Sicyos macrophyllus. Further work on breaking seed dormancy and increasing germination would produce more young plants for restoration to the mesic forest kīpuka. Seed dispersal studies might lead to understanding of the Park distributional pattern of the species. If alien grass control were undertaken at the Kīpuka Kī sites, post-treatment monitoring might reveal whether seedling recruitment was improved.

Zanthoxylum dipetalum var. *dipetalum*. Identification of insect species preying on natural seedlings would potentially allow treatments to be developed to protect them. Investigation of techniques to exclude Kalij pheasants from reproducing trees may provide information useful to managers. Seed dispersal studies may be warranted to confirm lack of seed dispersal.

Management Recommendations

- Effective control of alien rats during the periods of peak fruit production would likely benefit all studied species except *Z. dipetalum*.

- If an acceptable systemic insecticide is identified and approved for use in HAVO forests that does not unduly impact native insect populations, greater seed production might be achieved for the endangered *M. zahlbruckneri* and the rare *M. hawaiiensis*.
- Exclusion of Kalij pheasants from the vicinity of fruiting *Z. dipetalum* trees may be required if natural seedlings are to survive, and insecticidal treatment of natural seedlings may be warranted.
- Removal of alien grass cover from the areas adjacent to *S. macrophyllus* vines and beneath *H. giffardianus* may encourage seedling recruitment of these species, especially if combined with rat control.
- The continued propagation and planting of all five species in the two kīpuka and additional sites will augment populations with young plants, increase the number of populations, and improve chances of long-term survival of the species in HAVO.

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APPENDIX I

Descriptions of Flowers of the Five Study Species

Hibiscadelphus giffardianus. Flowers are borne singly on stout pedicels 1.5-3 cm long. The flower is subtended by 5-7 filiform involucre bracts 18-35 mm long that spread and reflex outward. The flower calyx is green, 2.7-3.7 cm long, and two to three parted or cleft. The curved flower corolla is 6-7 cm long with five petals, twisted together in a counter-clockwise direction, magenta and grey-green in color with star-shaped hairs on the outside. The interior of the corolla is magenta or dull red. Stamens are fused into a staminal column 7 to 8.4 cm long with many small anthers borne in the upper 1/3. The pistil is composed of a five-parted ovary and long exserted style with five branches, each topped with a terminal stigma covered with cream-colored to reddish hairs (Wagner *et al.* 1999, Baker and Allen 1977). The flower has no scent, but copious nectar is held in the base of the flower.

Melicope hawaiiensis. Flowers are unisexual and are borne together in inflorescences of three to 27 flowers. The inflorescence is an axillary cyme, sparsely to densely hairy, with a peduncle of variable length (3-30 mm) and pedicels 4-6 mm long. Individual flowers of the two sexes are externally similar in appearance. Female (pistillate) flowers are 5.5 mm long and 3 mm wide with four somewhat fleshy sepals, densely hairy and deltoid in shape. There are four petals, greenish-white and covered with short hairs on the outside and white or reddish and smooth (glabrous) on the inside. Stamens in female flowers are rudimentary, only 1.5 mm long, and the central pistil is composed of a superior, puberulent ovary and short style, and a reddish stigma with four lobes 0.7 mm long. Male (staminate) flowers are slightly larger, 6 mm long, and have sepals and petals similar to female flowers. Within male flowers there are eight stamens, four nearly 6 mm long and four shorter at 4 mm. All have anthers approximately 1 mm long. Male flowers have a non-functional central pistil with a short style (<0.5 mm) and reddish stigma. In both flowers a nectary disk with eight lobes surrounds the base of the ovary (Wagner *et al.* 1999, Stone 1969). Flowers have no noticeable scent.

Melicope zahlbruckneri. Flowers are unisexual and are borne together in small inflorescences of two to five flowers. The inflorescence is an axillary cyme, sparsely hairy, with a flattened peduncle 15-20 mm long and pedicels 4 mm long. Female flowers are somewhat smaller than males, with sepals 1.5 mm long and greenish-white petals 3 mm long. Female flowers have reduced stamens shorter than the petals and a central pistil with a smooth ovary

and short style 1 mm long tipped by a four-parted stigma. Male (staminate) flowers are smooth, with sepals 3.5 mm long and green to pink petals 6 mm long. Male flowers have eight stamens, four longer than the others and exserted from the flower; all have anthers 1.2 mm long. The reduced female organs of male flowers are a low ovary, style < 1 mm long, and a reddish stigma. Both sex flowers have an eight-lobed nectary disk surrounding the ovary (Wagner *et al.* 1999, Stone 1969). Flowers have no noticeable scent.

Sicyos macrophyllus. Flowers are unisexual, and male and female flowers are borne on separate inflorescences at the same leaf node. Male inflorescences are large panicles up to 25 cm long with peduncles of 6-20 cm. Pedicels of individual flowers are 3-10 mm long and are covered with glandular hairs. The male corolla (perianth) is greenish-yellow, 5 mm in diameter, and five-lobed. Within the male flower are three to five stamens coiled together, bearing anthers exserted from the corolla. Female (pistillate) flowers are borne in round heads with a peduncle of 2-8 cm. Wagner *et al.* (1999) report 2-6 pistillate flowers per head, but our monitoring revealed as many as 17 flowers per female inflorescence. Individual flowers are greenish-white, smaller in diameter than males, with five perianth lobes. No staminoides are present, and the central style with its three-lobed stigma is exserted from the flower (Wagner *et al.* 1999, St. John 1978).

Zanthoxylum dipetalum var. *dipetalum*. Flowers are unisexual and are borne on separate trees (dioecious). Flowers are clustered in open cymose inflorescences of five to fifteen with a peduncle 10-40 mm long and short pedicels 3-6 mm long. Inflorescences are typically terminal at branch tips, but may also be borne on stems opposite leaves. Flowers are creamy white, sometimes red-tinged, 6-10 mm long. Sepals are four in number, green, very small (1-1.5 mm), and ovate-deltate in shape. Flowers have two thick, fleshy petals, lanceolate to ovate in shape, with a small opening at the tip. Male (staminate) flowers have four stamens within the corolla and anthers 2-3 mm long at the tip; there is also a small, rudimentary central ovary. Female (pistillate) flowers are similar to males externally, but they lack stamens and have a central ovary with one or two short styles tipped with a round stigma (Wagner *et al.* 1999, Rock 1913). The flowers lack any scent or fragrance.

APPENDIX II

Greenhouse Seed Germination Trials for Four Study Species

Table 1. Greenhouse seed germination trials for *Hibiscadelphus giffardianus*.

Trial/Rep #	#Seeds	Date Sown	Date 1 st Germination	# Seeds Germinated	% Germinated	Date Terminated
1/1	25	5/2/06	5/18/06	16	64.0	1/23/07
1/2	25	5/2/06	5/18/06	2	8.0	1/23/07
1/3	25	5/2/06	5/18/06	7	28.0	1/23/07
1/4	25	5/2/06	5/18/06	11	44.0	1/23/07
1/5	25	5/2/06	5/18/06	5	20.0	1/23/07
1/6	25	5/2/06	5/18/06	6	24.0	1/23/07
1/7	25	5/2/06	5/18/06	7	28.0	1/23/07
5/1	26	2/4/08	2/11/08	3	11.5	8/6/08
5/2	26	2/4/08	2/11/08	4	15.4	8/6/08
5/3	26	2/4/08	n/a ¹	0	0	8/6/08
6/1	56	3/5/08	3/25/08	14	25.0	8/6/08
6/2	56	3/5/08	3/25/08	16	28.6	8/6/08
6/3	56	3/5/08	3/25/08	13	23.2	8/6/08
7/1	58	8/6/08	No data ²	11	19.0	2/20/09
7/2	58	8/6/08	No data	11	19.0	2/20/09
7/3	58	8/6/08	No data	12	20.7	2/20/09

¹ Not applicable, no germination observed.

² No data, date of first germination not recorded.

APPENDIX II (Continued)

Table 2. Greenhouse seed germination trials for *Melicope hawaiiensis*.

Trial/Rep #	#Seeds	Date Sown	Date 1st Germination	# Seeds Germinated	% Germinated	Date Terminated
1/1	10	9/26/06	3/8/07	2	20.0	1/8/08
1/2	10	9/26/06	n/a ¹	0	0	1/8/08
1/3	10	9/26/06	No data ²	1	10.0	1/8/08
1/4	10	9/26/06	No data	2	20.0	1/8/08
1/5	10	9/26/06	No data	2	20.0	1/8/08
1/6	10	9/26/06	No data	1	10.0	1/8/08
1/7	10	9/26/06	No data	1	10.0	1/8/08
1/8	10	9/26/06	n/a	0	0	1/8/08
1/9	10	9/26/06	No data	1	10.0	1/8/08
2/1	47	10/10/07	No data	2	4.3	3/13/09
2/2	47	10/10/07	No data	4	8.5	3/13/09
2/3	47	10/10/07	4/16/2008	4	8.5	3/13/09
3/1	72	8/12/08	n/a	0	0	ongoing
3/2	72	8/12/08	10/24/2008	1	1.4	ongoing
3/3	72	8/12/08	n/a	0	0	ongoing

¹ Not applicable, no germination observed.

² No data, date of first germination not recorded.

APPENDIX II (Continued)

Table 3. Greenhouse seed germination trials for *Zanthoxylum dipetalum* var. *dipetalum*.

Trial/Rep.#	#Seeds	Date Sown	Date 1st Germination	# Seeds Germinated	% Germinated	Date Terminated
5/1	30	11/21/06	2/6/07	14	46.7	6/18/07
5/2	30	11/21/06	2/6/07	20	66.7	6/18/07
5/3	30	11/21/06	2/6/07	17	56.7	6/18/07
6/1	50	7/2/07	9/4/2007	25	50.0	2/11/08
6/2	50	7/2/07	9/4/2007	28	56.0	2/11/08
6/3	50	7/2/07	9/4/2007	32	64.0	2/11/08
8/1	91	2/4/08	3/25/2008	41	45.1	2/2/09
8/2	91	2/4/08	3/25/2008	59	64.8	2/2/09
8/3	91	2/4/08	3/25/2008	51	56.0	2/2/09

APPENDIX II (Continued)

Table 4. Greenhouse seed germination trials for *Sicyos macrophyllus*.

Trial/Rep #	#Seeds	Date Sown	Date 1st Germination	# Seeds Germinated	% Germinated	Date Terminated
3/1	25	3/21/06	5/26/06	13	52	12/28/06
3/2	25	3/21/06	6/1/06	14	56	12/28/06
3/3	25	3/21/06	6/1/06	4	16	12/28/06
3/4	25	3/21/06	4/16/06	9	36	12/28/06
3/5	25	3/21/06	6/1/06	13	52	12/28/06
4/1	25	6/22/06	n/a ¹	0	0	No data
4/2	25	6/22/06	n/a	0	0	No data
4/3	25	6/22/06	n/a	0	0	No data
4/4	25	6/22/06	n/a	0	0	No data
5/1	100	6/30/06	7/11/06	4	4	7/18/06
5/2	100	6/30/06	7/8/06	9	9	7/18/06
5/3	100	6/30/06	7/7/06	4	4	7/18/06
5/4	91	6/30/06	n/a	0	0	7/18/06
8/1	25	6/27/07	7/16/07	7	28	7/18/08
8/2	25	6/27/07	7/16/07	9	36	7/18/08
8/3	25	6/27/07	7/16/07	8	32	7/18/08
9/1	25	6/27/07	7/16/07	7	28	7/18/08
9/2	25	6/27/07	7/16/07	5	20	7/18/08
9/3	25	6/27/07	7/16/07	9	36	7/18/08
10/1	25	6/27/07	7/16/07	6	24	7/18/08
10/2	25	6/27/07	7/16/07	3	12	7/18/08
10/3	25	6/27/07	7/16/07	7	28	7/18/08

¹ Not applicable, no germination observed.