



Technical Report HCSU-018

LIMITING FACTORS OF FOUR RARE PLANT SPECIES IN `ŌLA`A FOREST OF HAWAI`I VOLCANOES NATIONAL PARK

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March 2010

This product was prepared under Cooperative Agreement CA03WRAG0036
for the Pacific Island Ecosystems Research Center of the U.S. Geological Survey.

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SUMMARY

Three endangered or candidate endangered plant species native to `Ōla`a Forest (*Cyrtandra giffardii*, ha`iwale; *Phyllostegia floribunda*, a mint with no common name; and *Sicyos alba*, `ānunu) were studied for more than two years to determine their stand structures, short-term mortality rates, patterns of reproductive phenology, success of fruit production, seed germination rates in the greenhouse, presence of soil seed bank, and survival of both natural and planted seedlings. The role of rodents as seed predators was evaluated for *S. alba* using seed offerings in open and closed stations. A fourth endangered species (*Cyrtandra tintinnabula*) at a remote site in `Ōla`a was visited to determine its stand structure and mortality rate.

Cyrtandra giffardii displayed a stable population structure with many adults and few small or very large plants; the monitored population had a mortality rate of 7.3% over three years. Mortality of plantings from 2003-2004 in a re-introduced population of *Phyllostegia floribunda* was 21.4%. The stand structure of *C. tintinnabula* indicated a relatively stable population with both small and large plants present and a short-term mortality rate between visits of 14.5-17.0%. Four groups of *S. alba* vines were monitored; 3 of these have persisted in place for at least 15 years. All species monitored had annual patterns of flower and fruit phenology, although male inflorescences of *S. alba* showed a subannual pattern. Successful transition of flowers to fruit was high for *P. floribunda* (51.5%), moderate for *C. giffardii* (23.3%), and undetermined for *S. alba*. High percentage viability was demonstrated for seeds of *P. floribunda* and *S. alba* (78.5-100% positive to strongly positive in tetrazolium tests), but seed viability was not tested for *C. giffardii*.

Greenhouse germination rates were high for *P. floribunda* (88.0-92.0%), but variable and relatively low for *C. giffardii* (0-19.3%) and *S. alba* (4.0-11.1% in 2007 and 0 in 2008). No soil seed bank was detected for *S. alba* in 3 seasonal samplings, but *P. floribunda* was found to have a viable seed bank in April that persisted from at least the previous summer. Rodent predation of *S. alba* seeds was 93.3% in fruit offerings in accessible bait stations. Mortality of natural seedlings was high for both *P. floribunda* (90.2%) and *S. alba* (69.7%). Planted seedlings of *P. floribunda* produced flowers and fruit in their first year, and reproduction was higher in sunny plots than in shady plots. Mortality was high in both planting treatments, and

survival rates did not differ significantly in sun and shade ($\chi^2 = 0.48$, $df = 1$, $p = 0.490$). Three planted seedlings of *S. alba* survived for 12-16 months but did not reproduce.

Floral visitors were observed at *C. giffardii* and *P. floribunda* using digital video cameras and recorders. In almost 200 hours of observation, no visitors entered the flowers of *C. giffardii*, although one very small insect, either a micro-wasp (Hymenoptera) or fly (Diptera) was seen on the exterior of a corolla. In almost 300 hours of video observation, three floral visitors were identified at *P. floribunda* flowers. Honeybees (*Apis mellifera*) were likely pollinators, as they contacted both anthers and stigma of flowers. The mean visitation rate of honeybees was 0.003 visit/flower/hour, and visit duration ranged from 2 to 17 seconds. Fruit flies (Drosophilidae of undetermined species) crawled around flower interiors, but did not seem to forage for either nectar or pollen. Fruit fly mean visitation rate was 0.006 visit/flower/hour, and visit duration was 28 to 1,424 seconds. The third observed insect visitor was an endemic geometrid moth caterpillar (*Lophoplusia giffardi*), which was seen feeding on foliage and flowers of *P. floribunda*.

In conclusion, two of the three regularly-monitored rare plant species of `Ōla`a Forest appeared to have more than one limiting factor inhibiting the natural increase in their populations, while for *P. floribunda* the most important factor was high seedling mortality. Most plants of the monitored *C. giffardii* population appeared to be hybrids, probably with the more common species *C. lysiosepala*. Seed germination rates were low, and natural seedlings were not observed. Pollinators were not seen in many hours of observation, indicating that cross pollination is a rare or uncommon event. The re-introduced population of *P. floribunda* had relatively low mortality, and reproduction was successful with high rates of fruit formation from abundant flowers. Seed germination rates were high, and a soil seed bank was detected. Natural seedling recruitment was observed, but high seedling mortality indicated that this life stage was the most vulnerable in the species. The population of *S. alba* was small and the vine life form precluded an accurate estimate of the number of adult plants in `Ōla`a Forest. Natural dormancy was likely a factor in the observed low rate of seed germination. No soil seed bank was detected, and alien rodents were implicated as seed predators. Natural recruitment was observed at multiple sites in `Ōla`a, but seedling mortality was high. The cause of seedling mortality was not identified.

INTRODUCTION

Hawai'i Volcanoes National Park (HAVO), exclusive of the Kahuku Unit, supports approximately 400 native vascular plant species, and rare plant species comprise about 15% of the recorded flora (Higashino *et al.* 1988). Among the native plant species known from the park are 25 listed endangered and threatened plants, 6 candidates for endangered status, and 29 species of concern (U.S. Fish and Wildlife Service 2008, 2009). At least 8 endangered or candidate endangered plants and another 11 species of concern are currently found or reported to occur in `Ōla`a Tract, a montane rain forest with some of the highest native plant diversity contained within the park.

Approximately 30% of the park's total rain forest is now protected from feral pigs (*Sus scrofa scrofa*) by fenced exclosures (Katahira *et al.* 1993), including approximately half of `Ōla`a Tract. For the last 25 years HAVO resource managers have worked to remove feral pigs and the most invasive alien plant species from forests within the protected units of `Ōla`a, as well as from other wet forests designated as Special Ecological Areas (SEAs; Tunison and Stone 1992, Loh and Tunison 2009). Natural vegetation within the managed units of `Ōla`a has responded positively (Loh and Tunison 1999), but most of the endangered and rare plant populations have not increased in size or expanded their ranges (Pratt and Abbott 1997) despite decades of protection from feral animals and reduction of invasive alien plants. Approximately half of the endangered species and species of concern of `Ōla`a Forest were recently targeted for augmentation or re-introduction in a rare plant stabilization project (Belfield, unpublished). If the factors causing the rarity and low reproduction of these plant populations were better understood, the success of the park's stabilization program might be enhanced and additional tools might be developed to better manage the park's endangered species in their natural habitats. Determination of the causes of reproductive failure of both extant and restored rare species may allow managers to stabilize remaining rare plant populations and prevent further losses.

Two endangered plant species with known populations in the western, more accessible half of `Ōla`a (*Cyrtandra giffardii* and *Sicyos alba*), along with a newly re-introduced population of a candidate endangered species in the same area (*Phyllostegia floribunda*), were selected for a limiting factors study. Another endangered species (*C. tintinnabula*), recently discovered in

the remote northeastern quarter of `Ōla`a, was visited repeatedly to determine its population size, stand structure, and survival rate.

Cyrtandra giffardii, ha`iwale, an endangered species, is endemic to windward Hawai`i Island, where it is found in only three areas: Kīlauea, Kūlani, and Laupāhoehoe (U.S. Fish and Wildlife Service 1996, Wagner *et al.* 1999). The type locality is Kīlauea near the Volcano House (Rock 1919), but the species has not been collected there since the 1930s (Wagner *et al.* 1999). In HAVO, this *Cyrtandra* seems to be currently restricted to western `Ōla`a, although a recognized hybrid for which *C. giffardii* is a presumed parent (*C. ramosissima*) has been found in forests of Kīlauea's East Rift (Fosberg 1966, Belfield 1998). *Cyrtandra giffardii* is known to hybridize with other more common members of the genus (Wagner *et al.* 1999). In the early 1990s, approximately 90 individuals thought to represent this endangered species were found on systematic transects of `Ōla`a; most of them were concentrated in the western half of the fenced Koa Unit (Pratt and Abbott 1997). It is likely that many of the individuals found in the 1992-1994 survey were actually hybrids. *Cyrtandra giffardii* is a small, soft-wooded tree or shrub with many branches and relatively small opposite leaves. It is distinguished from other members of its genus by its few-flowered inflorescences on long slender stalks, relatively small white flowers with short and narrow calyx lobes, and small round white berries. A more detailed description of the flowers of this and two other study species is found in Appendix I.

Cyrtandra tintinnabula, ha`iwale, also an endangered species, is endemic to windward Hawai`i Island and until recently was known only from the Laupāhoehoe region of Mauna Kea (U.S. Fish and Wildlife Service 1996, Wagner *et al.* 1999). In 2001, a small population of this species was found growing in sites protected from feral pigs at a trench-like geological feature in the unfenced northeastern corner of `Ōla`a Forest (Waite and Pratt 2007). This species is a weak-stemmed shrub of low stature (1-2 m) with broad, asymmetrical leaves covered with yellowish hairs. The white flowers are borne in congested, axillary inflorescences with large leaf-like bracts. Individual flowers are small, covered with hairs, and have distinctive triangular (deltate), reflexed (curved backward) calyx lobes (Rock 1918, Wagner *et al.* 1999).

Phyllostegia floribunda, a candidate endangered species with no common name (U.S. Fish and Wildlife Service 2008, 2009), is 1 of 11 endemic mint species found in the original section of HAVO (exclusive of the Kahuku Unit addition); most of these occur in rain forest habitat (Higashino *et al.* 1988). Endemic to Hawai`i Island, this mint is distributed over four

disjunct areas of the island including wet forests between Kīlauea and Laupāhoehoe (Wagner *et al.* 1999). The species was first collected on the island near Kealakekua by David Nelson in 1779 (St. John 1978a), and it has also been known as *P. forbesi* and *P. villosa* (St. John 1976). In HAVO, *P. floribunda* has been found naturally at a single site in `Ōla`a (Pratt and Abbott 1997) and at two sites in Kīlauea's East Rift near Nāpau Crater (Belfield 1998) and in a kīpuka near Pu`u `Ō`ō (Higashino and Katahira #9800, 1982, HAVO Herbarium). As part of a HAVO rare plant stabilization project, plants were propagated from material collected in the Waiākea Forest Reserve, and more than 160 individuals were planted at several sites in `Ōla`a (Belfield, unpublished). *Phyllostegia floribunda* is a weakly woody shrub with opposite, ovate, hirsute leaves. Its axillary inflorescences are covered with glandular hairs and bear tubular, red to maroon flowers, which are unusual among the predominantly white-flowered members of this genus. Fruits are four-parted fleshy nutlets, which are shiny black when mature (Sherff 1935, Wagner *et al.* 1999).

Sicyos alba, `ānunu, is a listed endangered species (U.S. Fish and Wildlife Service 1997) among 14 endemic Hawaiian members of a genus of vines widely distributed in America, Australia, and the Pacific (Wagner *et al.* 1999). Endemic to Hawai`i Island, *S. alba* is historically known from Mauna Kea, but has been recently observed only in Pu`u Maka`ala Natural Area Reserve, `Ōla`a Forest Reserve, and HAVO `Ōla`a Tract (U.S. Fish and Wildlife Service 1997). The vine has also been known by the name *Sarx alba* (St. John 1978b). *Sicyos* without flowers or fruit was collected in the park's `Ōla`a Tract in 1974 (Jacobi and Warshauer, unpublished) and again in 1989 within forest later included in the fenced Koa Unit (T. Tunison, pers. comm.). Subsequently, the *Sicyos* of `Ōla`a Forest was observed with flowers and fruit and was identified as *S. alba*. A systematic survey of five units of the park's `Ōla`a Forest located four sites supporting the species (Pratt and Abbott 1997); a fifth `Ōla`a site was discovered in 2000. *Sicyos alba* is a robust, fleshy-stemmed vine with broad, three- to five-lobed leaves and male and female flowers borne in separate inflorescences at the same leaf nodes. Male flowers are small with five white corolla lobes and exerted, curved anthers. Female flowers are tubular with four white lobes and an inferior ovary that develops into a fleshy, indehiscent, pale green to white fruit shaped like a small cucumber (St. John 1978b, Wagner *et al.* 1999).

The objectives of this study were: 1) determine population size and stand structure for three rare plant species of `Ōla`a Forest and to evaluate the success of a planted population of *P. floribunda*; 2) measure flower and fruit production through monthly or bimonthly monitoring of phenology of three species; 3) investigate the cause of reproductive failure by following the fate of a sub-sample of flowers and fruit and identifying potential pollinators; 4) determine the success of seedling recruitment by quantifying seed germination rates and soil seed banks and following seedling establishment; and 5) determine the role of rodents in seed predation for *S. alba*.

METHODS

Description of the Study Area

`Ōla`a Forest tract is composed of two parcels of land that are not contiguous with the boundaries of the rest of Hawai`i Volcanoes National Park (Fig. 1). `Ōla`a Forest is in the Puna District, approximately 2-3 km northeast of Kīlauea Caldera; portions of Volcano Village and ranchland separate the forest from the rest of Hawai`i Volcanoes National Park. The large tract of `Ōla`a is an approximately square parcel, 6 km on each side; its area is 3,765 ha. The small tract is adjacent to the southwest corner of the larger parcel across Wright Road, at approximately 1,160 m (3,800 ft) elevation. Slightly larger than 1 km square, this unit contains 144 ha (National Park Service 1985). For the convenience of the reader using topographic maps with contour intervals delineated in feet, English equivalents are given for reported elevations.

The Koa Unit enclosure is in the southwestern corner of the large tract of `Ōla`a Forest between 1,070 (3,510 ft) and 1,220 m (4,000 ft) elevation (Fig. 1). The enclosure is approximately 3 km wide (east to west) and 2.5 km long (roughly north to south) and extends eastward to include a kīpuka of *Acacia koa* (koa) trees, after which the unit is named. The area included within the fenced enclosure is approximately 1,000 ha (Loh and Tunison 1999). Lands adjacent to the Koa Unit study area are the managed Agriculture ("Ag.") Unit to the north and the unfenced part of `Ōla`a Forest to the east. Private ranch land and residential parcels abut the Koa Unit to the south and surround the small tract.

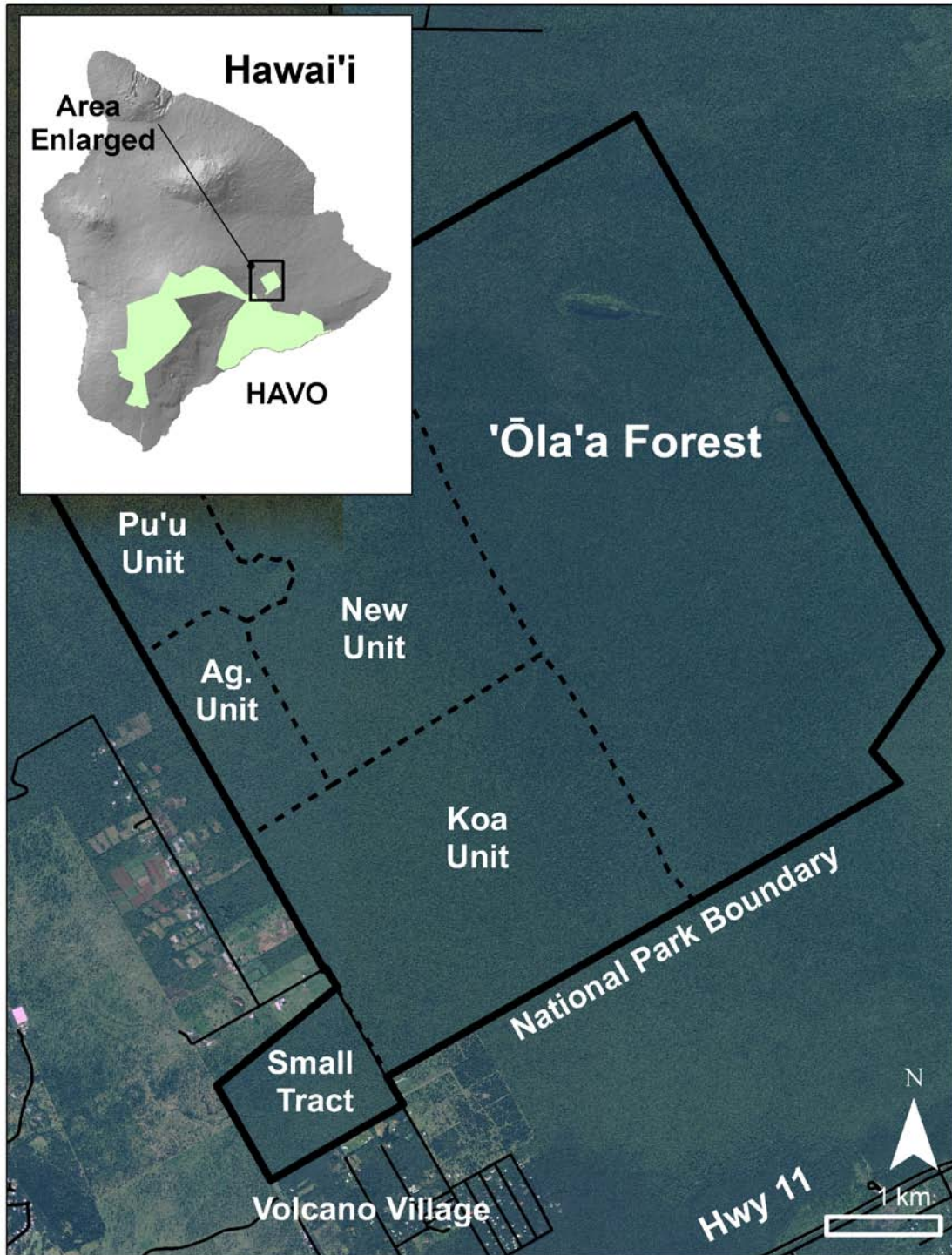


Figure 1. The study sites of Koa Unit and Small Tract and other management units in 'Ōla`a Forest, Hawai`i Volcanoes National Park (HAVO).

Geology and Soils

The large tract of `Ōla`a Forest (and about half of the small tract) is on the eastern slope of Mauna Loa Volcano near its contact with Kīlauea Volcano. Most of the substrate of `Ōla`a is classified as the Kahuku volcanic series overlain by Pāhala ash; the younger Ka`ū volcanic series is also found within the tract (MacDonald *et al.* 1983). In a recent geological map of Hawai`i Island, most of the `Ōla`a substrates in the area enclosed by the Koa Unit and small tract were dated at 5,000-10,000 years before present (Wolfe and Morris 1996). The park's `Ōla`a Forest is growing on relatively old surfaces of Mauna Loa (Lockwood *et al.* 1988); about 90% of the volcano is covered by flows <4,000 years old, and 40% of Mauna Loa has been covered by lava in the last 1,000 years (Lockwood and Lipman 1987).

At least seven soil series have been recognized within the park's `Ōla`a Forest. All of the small tract and the western half of the Koa Unit were mapped as Puaulu silt loam, a deep, well-drained soil formed in layers of volcanic ash. The eastern part of the fenced Koa Unit enclosure was mapped as Akaka silty clay loam; this soil is well-drained, typically achieves a depth of 183 cm, and is also derived from volcanic ash (Sato *et al.* 1973).

Climate

`Ōla`a Forest is in the montane wet zone with relatively cool temperatures and no pronounced dry season (Doty and Mueller-Dombois 1966). Mean annual rainfall in the `Ōla`a region is 3,000-4,000 mm, with wetter conditions to the east. At the nearby Wright Road farm lots, mean annual precipitation is 2,899 mm. The wettest months are in the winter and spring (November to April); summer months (June to September) are drier, but monthly mean rainfall does not drop below 100 mm in any month (Giambelluca *et al.* 1986). Mean annual temperature in the `Ōla`a area falls between 15.6 and 18.3 °C (Hawaii State Department of Land and Natural Resources 1970). Daily temperature highs and lows are greater during summer months but do not vary greatly throughout the year (Jacobi and Warshauer, unpublished). On the windward slopes of Mauna Loa, the `Ōla`a region receives the prevailing northeast trade winds for much of the year. High winds are an occasional winter phenomenon; wind storms may result in heavy foliage loss and tree falls in `Ōla`a (Gerrish 1980).

Vegetation and History of Management

The vegetation of the current study area was mapped primarily as open *Metrosideros polymorpha* (‘ōhi‘a lehua) forest with an understory of native trees, tree ferns, and native shrubs. The eastern part of the Koa Unit was mapped as scattered *Metrosideros* with snags, indicating ‘ōhi‘a dieback (Jacobi, unpublished; Jacobi and Warshauer, unpublished). The most common native trees in the understory of ‘Ōla‘a Forest are *Cheirodendron trigynum* (‘ōlapa), *Ilex anomala* (kāwa‘u or Hawaiian holly), *Myrsine lessertiana* (kōlea lau nui), and *Perrottetia sandwicensis* (olomea). A distinct understory layer of hāpu‘u tree ferns (*Cibotium glaucum*, *C. menziesii*, *C. chamissoi*) is prominent beneath the secondary tree layer, and native shrub species, such as *Broussaisia arguta* (kanawao or pū‘ahanui) and *Vaccinium calycinum* (‘ōhelo kau la‘āu) are common. The ground cover is variable but is usually composed of native terrestrial ferns, tree seedlings and saplings, native herbs, such as *Peperomia* spp. (‘ala‘alawainui), and a suite of herbaceous alien plants.

‘Ōla‘a is a recognized site of *Metrosideros* or ‘ōhi‘a dieback and contains a number of long-term study plots (Jacobi *et al.* 1983). The dieback in ‘Ōla‘a was classified primarily as “displacement dieback,” where large *Metrosideros* trees die and *Cibotium* tree ferns become the dominant plant (Mueller-Dombois *et al.* 1977); “wetland dieback,” characterized by replacement of dead trees by young *Metrosideros*, was also described in the region.

Feral pigs, *Sus scrofa scrofa*, have been long recognized as a serious disruptive influence in Hawaiian rain forests (Stone 1985, Stone *et al.* 1992). Systematic feral pig control began in the park in 1980 (Hone and Stone 1989). ‘Ōla‘a small tract was the first of five managed units in ‘Ōla‘a to be fenced to exclude pigs, and the enclosure has been essentially pig-free since 1981 (H. Hoshida, pers. comm.). The Koa Unit was fenced in 1989, and pigs were removed from the enclosure by 1994 (Loh and Tunison 1999).

Control of the most invasive alien plants began in the small tract in 1985 (Tunison 1992, Tunison and Stone 1992) and was later expanded to the western half of the Koa Unit (Loh and Tunison 2009). Highly invasive species of the managed areas, such as *Rubus ellipticus* var. *obcordatus* (yellow Himalayan raspberry), *Passiflora tarminiana* (banana poka), *Hedychium gardnerianum* (kāhili ginger), and *Psidium cattleianum* (strawberry guava), have been periodically treated using chemical and mechanical methods. In the period between 1998 and

2008, protected units of `Ōla`a were used as restoration and augmentation sites for at least 12 rare plant species known from this rain forest (Belfield, unpublished).

Sampling Methods

Size Class Structure, Growth, and Mortality

For *Cyrtandra giffardii*, all previously mapped individuals in the first 1,000 m of three transects in the Koa Unit and the fence line between the Koa Unit and the adjacent Agriculture Unit (Pratt and Abbott 1997) were revisited in January 2006. Those that were fertile and appeared to have characteristics of *C. giffardii* were given a number and marked with a colored flagstick. We also searched the immediate area of relocated individuals to look for additional members of the species. Height was measured at the tallest point to the nearest cm and basal diameter was measured with a diameter tape. Height was repeatedly measured during monitoring periods over the next two years, and final height and diameter measurements were made in August 2008.

The only known site supporting *C. tintinnabula* (Waite and Pratt 2007) was visited in July 2006 and plants in the secondary crater of the trench-like feature were relocated. Thirty-five plants were found, tagged, and had their height measured at the tallest point. Because of time limitations, not all plants were tagged and measured, but on a second visit in November 2006, all additional plants within the secondary crater were tagged and measured and evaluated for condition. One visit was made in 2007, when plants were re-measured and mortality was recorded.

Phyllostegia floribunda individuals monitored were propagated and planted in 2003 and 2004 as part of a HAVO Resources Management Division rare plant stabilization project (Belfield, unpublished). All plants in good condition at two planting sites were selected for this study; the sites monitored were on transect 18 in Koa Unit (24 plants) and on transect 3 of the small tract (18 plants). The tallest shoot was measured at each monitoring period between September 2005 and September 2008; the number of live basal shoots was also periodically counted. Mortality was evaluated at each monitoring period.

The phenology of *Sicyos alba* was monitored at the four known sites for the species in `Ōla`a Forest, but because of the vine growth form of this species we were unable to discern individuals or determine growth in height (other than for seedlings).

Phenology and Success of Flower and Fruit Production

For *Cyrtandra giffardii*, all buds, flowers, immature fruit, and mature fruit were counted on each individual plant (55) at bimonthly monitoring periods. Reproductive success was evaluated by tagging each inflorescence of each fertile plant with a colored wire or a numbered twist tie and recording the number of buds, flowers, immature fruit, and mature fruit present within each inflorescence when the inflorescence was first noted and at subsequent monitoring periods. Phenology of *C. tintinnabula* was not monitored because of the remoteness of the population in `Ōla`a.

Phenology of 42 *Phyllostegia floribunda* plants was monitored monthly from December 2006 to September 2008 except for several months (February and August 2007, February 2008) that were missed because of scheduling conflicts. All buds, flowers, immature green fruit, and mature fruit (black or enlarged and dark green) were counted on each individual of the original plantings at two sites. The successful transition of *P. floribunda* from flower to fruit was evaluated during the season of peak flowering in June and July 2008. Equal numbers of flowers (103) and small, green, developing fruit (103) were individually tagged with numbered twist ties on 14 of the monitored *P. floribunda* plants; approximately half of the tagged plants were at each of the large and small tract sites. Plants were visited at two-week intervals to monitor the successful development of immature and mature fruit. Empty expanded calyxes were interpreted as dispersed mature fruit.

Sicyos alba phenology was monitored monthly or bimonthly at three of the four known sites supporting this species in `Ōla`a Koa Unit; the fourth site had only seedlings. In September 2006, six observation points were established on the edge of the largest *S. alba* patch where the vine could be easily seen and fruits were obvious on the ground beneath the vine. The point was marked on the ground and the azimuth of the field of view with live vine was recorded. Three observation points were also established at each of the other sites supporting adult vines. At each monitoring period, all inflorescences were counted in the field of view at the established direction; categories of inflorescences counted were male flowers or

buds, female flowers or buds, small green fruit, and large green or pale fruit (mature). The number of fruit on the ground in a square meter area at observation points was also counted. Binoculars (Leica 8 x 32) were used for phenology observations. We attempted to tag inflorescences and determine successful transition rate to fruit, but few inflorescences were reachable and even fewer remained at the subsequent monitoring period, so transition rates of flowers to fruit were not obtained for this species.

Pollination Studies—Floral Visitor Observations

Composition of the floral visitor community and the rates of visitation were quantified by monitoring insect activity at adjacent flowers of *Cyrtandra giffardii* and *Phyllostegia floribunda*. Observations were made by video, using digital video cameras (Super Circuits PC219ZWPH) mounted on tripods. The video was recorded onto a digital video recorder (Lorex DXR4280UQA) at a rate of 2 frames/second to conserve digital memory. The entire unit was powered by 12-volt deep cycle marine batteries. For each floral visitor, the following data were recorded: identity of the visitor to the lowest possible taxonomic level allowed by the video recording (see Acknowledgements for entomologists consulted), whether or not it entered the corolla, the mode of entry into the corolla, and the duration of the visit.

For *C. giffardii*, recordings were performed on one to two flowers continuously throughout the daytime and into the night for 12 days spanning late October to late November 2007, totaling 270.4 hours of observation. For *P. floribunda*, one to eight flowers were recorded continuously between 0630 and 1900 for 15 days from late June to early July 2007, totaling 298.0 hours of observation.

Cyrtandra giffardii and Hybrid Morphology and Density in `Ōla`a

Because it seemed likely that the group of *C. giffardii* plants we were sampling for phenology included hybrids with other species of *Cyrtandra*, we collected quantitative data on morphology to allow us to objectively distinguish between the endangered species and its hybrids. We measured a set of inflorescence and leaf characteristics of 61 monitored plants at least three times between January and July 2008 and again in January 2009. We selected a suite of characters to measure based on published values in Wagner *et al.* (1999), which should not overlap in the endangered *C. giffardii* and the more common *C. lysiosepala* found at the

study site. For bract and calyx length, we expanded the likely *C. giffardii* length beyond the published measurements to 1.0 cm (10 mm) because we noted that these structures shrank when dried, and it is likely that published character measurements were derived from pressed and dried plant specimens. All inflorescences measured were in the stage supporting flowers, immature fruit, fruit, or a combination of structures. Leaves were measured on fertile branches when possible and were those with inflorescences in their axils; on sterile plants, leaves measured were the uppermost apparently full-size leaves. Qualitative characteristics of leaf pubescence, leaf shape, and calyx lobe shape were also recorded.

In order to evaluate the relative density of the target *C. giffardii* or hybrids and *C. lysiosepala* in the study area of `Ōla`a Koa Unit, we randomly selected three plots along the first 1,000 m of the transect that supported the highest number of monitored *C. giffardii* (transect 18). Random numbers were selected along the existing transect, and a coin was flipped to determine on which side of the transect the plot should be placed. Plots were 20 x 20 m in size (400 m²). All plants of *C. giffardii*, *C. lysiosepala*, and apparent hybrids were counted within each of the three plots.

Seedlings

Natural seedlings. Natural seedlings were detected at the original planting of *Phyllostegia floribunda* and at all four monitored sites with *Sicyos alba*. In both cases, seedlings were marked with a numbered pot tag and revisited at monthly or bimonthly periods in 2006-2008, when their height was measured and vigor evaluated. Vigor was considered good if seedlings looked normal and fair if they appeared less than fully healthy. Seedlings were rated poor if they were yellow or brown, had few leaves, or were wilted. Final counts of seedlings were made in May 2009.

Planted seedlings. Seedlings of *Phyllostegia floribunda* obtained from early germination trials were planted on transect 19 in large tract within three pairs of plots 10 x 10 m in size. Plot pairs were placed at randomly selected points in the first 200 m of the transect. Treatment and control plots were separated by a distance of 10 m. Expanded tree fern fronds leaning over the treatment plot were removed from one of each pair of plots (sun plots), and tree ferns were not manipulated at adjacent control plots (shade plots). In May 2006, seven seedlings were planted in each of the six plots. When seedlings were available from later germination

trials, six additional seedlings were planted in each plot in April 2007; expanded tree ferns were again removed at the second planting. The height of seedlings was measured at planting and at subsequent monitoring periods through August 2008. Plots were revisited in May 2009 to evaluate mortality of planted seedlings. The phenology of seedlings planted in sun and shade plots was monitored bimonthly or monthly from April 2007 until August 2008. As with the earlier plantings, all buds, flowers, immature fruit and mature fruit were counted on each individual plant.

Three *Sicyos alba* seedlings derived from seed germination trials were planted by HAVO Natural Resources Management Division staff in May 2007 at a sun plot used for *P. floribunda* seedling plantings on transect 19 of the Koa Unit. Height and survival of seedlings were monitored monthly or bimonthly for 15 months.

Seeds and Seed Germination

Field seeding in sun and shade plots. When *P. floribunda* seedlings were planted in sun and shade plots in May 2007, a 1 x 1 m² plot was established at a randomly selected point on the edge of each plot. Within each plot, 50 fresh, cleaned seeds were planted just below the surface in rows of 10 seeds each. Seed plots were examined for germination at monthly monitoring periods.

Soil seed bank. The soil seed bank was not examined for *C. giffardii* because the seeds of this species are too tiny to detect by sifting soil samples, and seedling germination in greenhouse-sowed seeds is slow and highly variable. The soil seed bank was sampled for *P. floribunda* in April 2009 by collecting five soil cores from the area within a 1-m radius around each of five large plants that had flowered and fruited in 2008. Soil cores were planted in flats over a base of cinder and perlite in the HAVO Natural Resources Management greenhouse; flats received misting three times a day. The elevation and climate at the greenhouse are similar to those of `Ōla`a Forest. Planted flats were monitored weekly for four months, and all seedlings that appeared were identified and counted. The soil seed bank at the largest patch of *S. alba* was sampled in February and April 2008 and January 2009. In the first two samplings, two soil cores were collected at each of five observation points where seeds had previously been seen on the ground. At the last sampling, soil cores were collected at five sites beneath live vine.

Soil samples were sifted to extract any seeds or fruit, whole or partial. The soil coring device for both *P. floribunda* and *S. alba* was a bulb planter 6 cm in diameter and 10 cm long.

Seed predation experiment. In January 2009, three sets of rat bait stations (Protecta brand black plastic boxes) were placed at the largest *S. alba* group. Pairs of stations were placed at phenology observation points and were at least 10 m apart. Ten fresh green mature *S. alba* fruits were placed in each of three open bait stations and adjacent stations with openings covered with rodent-excluding screen. The seed offerings were visited monthly for three months, seeds were counted, and signs of feeding by rodents or insects were noted.

Seed germination in the greenhouse. Seeds of *Cyrtandra giffardii*, *Phyllostegia floribunda*, and *Sicyos alba* were collected from multiple plants several times throughout the study period. No more than 10% of the standing seed crop was collected. Germination trials used fresh seeds whenever possible, but sometimes seeds were stored in a refrigerator for up to four months until an adequate sample of seeds could be collected. No special treatment was given to any seeds other than *Sicyos alba*, which in some trials had the enclosing fruit wall nicked using clippers. Seeds were planted in replicated flats or pots filled with a potting medium of equal parts vermiculite, perlite, and cinder; *C. giffardii* pots were topped with fine, sifted cinder to prevent the tiny seeds from slipping down into the basal coarse medium. All germination trials were carried out in the HAVO Natural Resources Management greenhouse located in rain forest habitat near Kīlauea `Iki Crater at 1,170 m (3,840 ft) elevation. Flats were misted with water three times a day. The time of first germination was noted for some trials, and counts of seedlings were made weekly. Trials were terminated when seedlings ceased to appear or after 8 to 24 months with no germination.

For *C. giffardii*, the first germination trial was carried out with 50 seeds counted from the fruit pulp and germinated on filter paper in each of two Petri dishes. Two subsequent trials in 2006, both with three replicates, used 50 seeds counted from the fruit of one plant in each trial, surface sowed on replicate pots with a medium of vermiculite, perlite, and fine cinder. In 2007 and 2008, 25 separate fruit were collected from 10 different plants and individually sown in separate pots. *Cyrtandra* berries were squashed on a filter paper to remove pulp, and seeds of *C. giffardii* were washed off the filter paper directly into pots covered with a top layer of fine sifted cinder. Seeds were counted for a sample of 13 berries using a dissecting microscope, and the mean number of seeds per fruit was used to calculate germination rate for those fruits

without direct seed counts. Petri dish trials were followed for two months, and trials in 2006 were monitored for eight months. In 2007 and 2008, trials were terminated after 12 to 24 months of monitoring.

Three germination trials were carried out on fresh *P. floribunda* seeds in the HAVO greenhouse. Seeds were removed from the flesh of the fruit and air-dried. The first trial was sown in July 2006, and the others were sown in June and July 2008. Germination trials used from 25 to 56 seeds in replicated pots or flats. Trials were monitored for six to nine months before termination.

Following preliminary trials to test propagation techniques and media, four germination trials were accomplished with *S. alba* seeds. Fruit (each containing one seed) had the flesh cleaned off but retained the tough inner fruit wall; these were air-dried and were stored for one to four months before planting. Two trials used seeds/fruit with no treatment, and two trials used fruit treated by clipping the inner fruit wall to the enclosed seed. Germination trials used from 18 to 33 seeds in each of three replicated flats. Trials were monitored for 7 to 12 months.

Seed viability tests. To complement germination trials and estimate seed viability, a tetrazolium test was used on ungerminated seeds of *P. floribunda* (18) and *S. alba* (20). Seeds were prepared by opening the seed coat and bisecting the endosperm and embryo with a razor blade. The internal parts of the healthy seeds were not removed from the bisected halves of the seed coat before treatment. Prior to opening, the seeds of both species were first imbibed in tap water for 24 hours. After soaking, the remnants of fibrous fruit material were carefully scraped off the seed coat of each seed using a razor and dissecting microscope. Each seed was individually treated with a 0.1% solution of 2,3,5-triphenyl tetrazolium chloride in a buffer of 66 mM KH_2PO_4 and 33 mM Na_2HPO_4 (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 a compound called formazan, which appears red and stains healthy tissues (Cottrell 1947, Baskin and Baskin 1998).

Results of the tetrazolium tests were recorded approximately 24 hrs after treatment. Each seed was individually inspected for staining. A dissecting microscope was used to accurately locate and inspect the embryo. Viability was assessed by assigning each seed to a category representing the level of staining that occurred 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 one of two methods. For data recorded as categories, including rodent seed predation, 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 and number of stems, means were compared using simple two-sample t-tests assuming unequal variance. The t-tests were run using the Analysis ToolPak in Excel (Microsoft 2003).

Phenology patterns of *Phyllostegia floribunda* plants were compared between sun and shade treatments using a repeated measures analysis of variance (ANOVA). 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: Autoregressive (1) (AR1), Compound Symmetry (CS), Toeplitz (TOEP), and Unstructured (UN). The results of comparisons using AICc (corrected Akaike information criterion) values indicated that for bud data AR1 was best fit, while for flowers, immature fruit, and mature fruit, UN was the best fit variance-covariance structure. All reported values come from output generated by models with the best fit variance-covariance structure. Denominator degrees of freedom were adjusted using the Kenward-Roger adjustment statement. The data contained a large number of zeros associated with periods where some plants were not producing, so count data were $\ln(\text{count}+1)$ 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 pollination studies of *C. giffardii* and *P. floribunda*, mean visitation rates for each floral visitor species were calculated as the total number of flowers visited per total number of flowers observed per hour and were based on an average across all observation recordings. Mean visit duration was calculated as the total time spent visiting a flower and averaged across individual visits.

RESULTS

Cyrtandra giffardii, Ha`iwale

Size Class Structure, Growth, and Mortality

Cyrtandra giffardii plants were found in the `Ōla`a Koa Unit using information from a previous study (Pratt and Abbott 1997). All plants that appeared to represent this endangered species encountered in the study area were included in the sampled population (Fig. 2). The population was composed mostly of plants between 50 cm and 2 m in height; few small plants or trees >2 m tall were found in the study area (Fig. 3). The differences in stand structure at the beginning (2006) and end of the study (2008) indicated that some of the plants in the height class >50 to 100 cm increased in stature and moved into the height classes of >100-150 cm and >150-200 cm.

The mean growth in height of 49 plants measured in both 2006 and 2008 was 29.7 cm (\pm 30.6 SD). Heights and basal diameters of *C. giffardii* plants increased during the monitoring period. The mean height of 53 plants was 118.3 cm (\pm 52.7 SD) in 2006 and that of 59 plants was 140.1 cm (\pm 51.4 SD) in 2008. The survivors of the original 53 plants were included within the group of 59 measured in 2008. The mean basal diameter of monitored plants was 19.1 mm (\pm 9.3 SD, n = 50) in 2006 and 21.5 mm (\pm 8.3 SD, n = 50) in 2008.

The mortality of 55 plants consistently monitored was low; only 4 plants (7.3%) died during the three-year study. In all cases, death was caused by fallen trees or branches, which broke the main stems of the *C. giffardii* plants or completely buried them.

Reproductive Phenology 2006-2008

Most of the sampled *C. giffardii* plants were fertile during more than two years of phenology monitoring. Buds were produced by a few plants at each bimonthly monitoring, but peaks in bud production were noted in February and November when up to 67.9% of plants were seen with buds. Flowers were observed on fewer plants, and the greatest proportion of sampled plants produced flowers during the period from November to May (Fig. 4). Seasonality was more clearly exhibited by the plant means for buds and flowers. In the two years for which

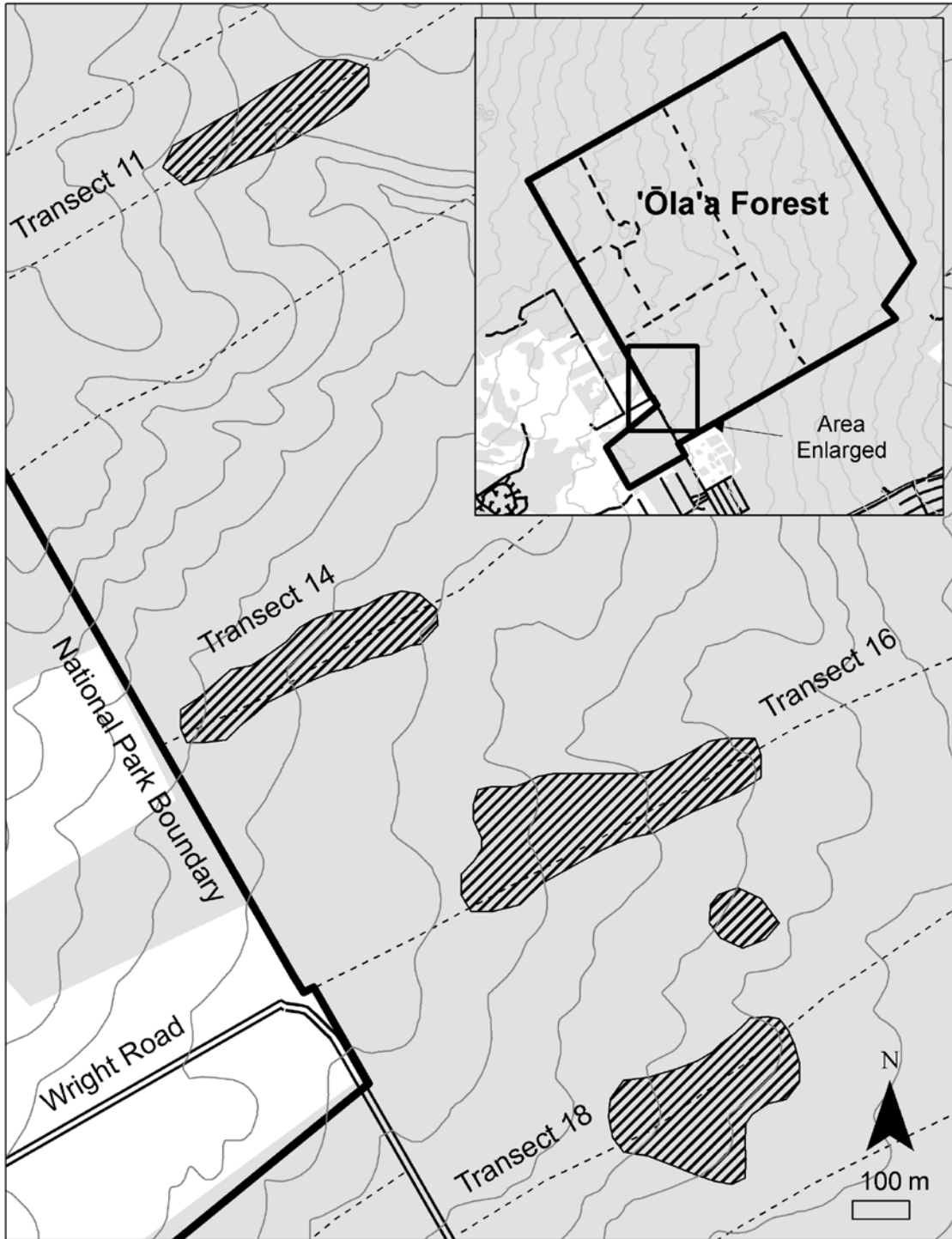


Figure 2. Location of *Cyrtandra giffardii* plants and likely hybrids (striped areas) in the 'Ōla'a Forest Koa Unit study area, HAVO.

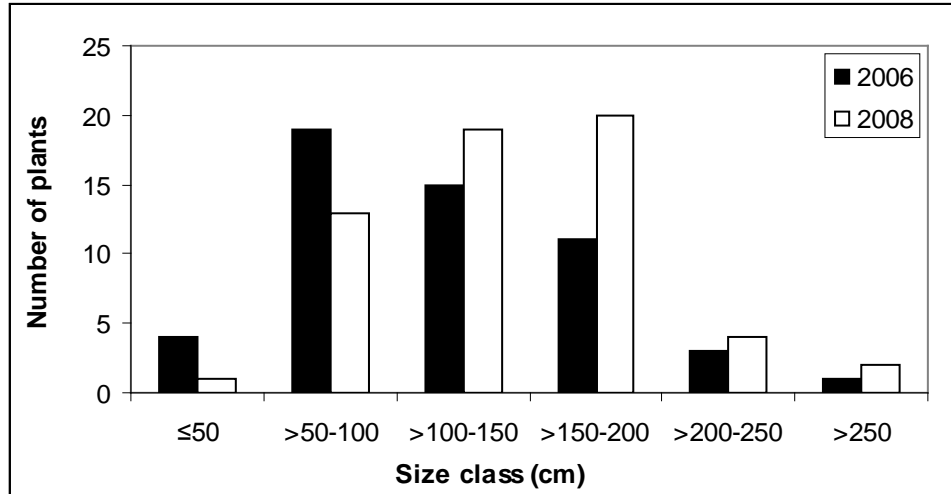


Figure 3. Size class structure of *Cyrtandra giffardii* plants in `Ōla`a Koa Unit, HAVO, in 2006 and 2008.

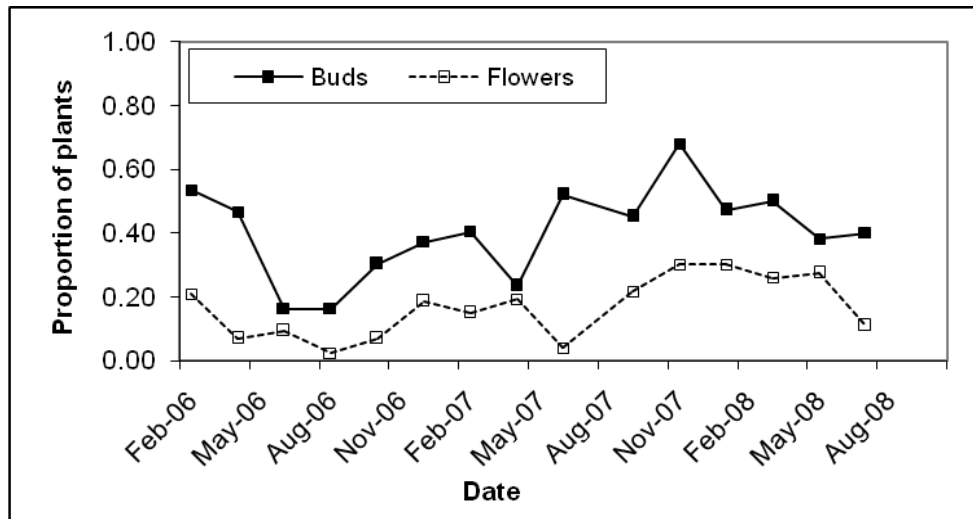


Figure 4. Proportion of *Cyrtandra giffardii* plants producing buds and flowers in `Ōla`a Koa Unit, HAVO, 2006-2008.

we have complete data, the mean number of buds was highest in December 2006 (6.3 ± 11.4 SD) and November 2007 (11.1 ± 17.3 SD) (Fig. 5). Flowers were far less abundant than buds with peak means <1 per plant, but a similar pattern of greatest production in the period from November to April was displayed (Fig. 6). We did not observe predation to buds and have no

data on the cause of the failure of most buds to form flowers. The two-month interval of monitoring may have been too long to adequately detect the transition of buds to ephemeral flowers, although the monitoring interval appeared to be sufficient to determine the successful transition of buds to longer-lasting immature fruit.

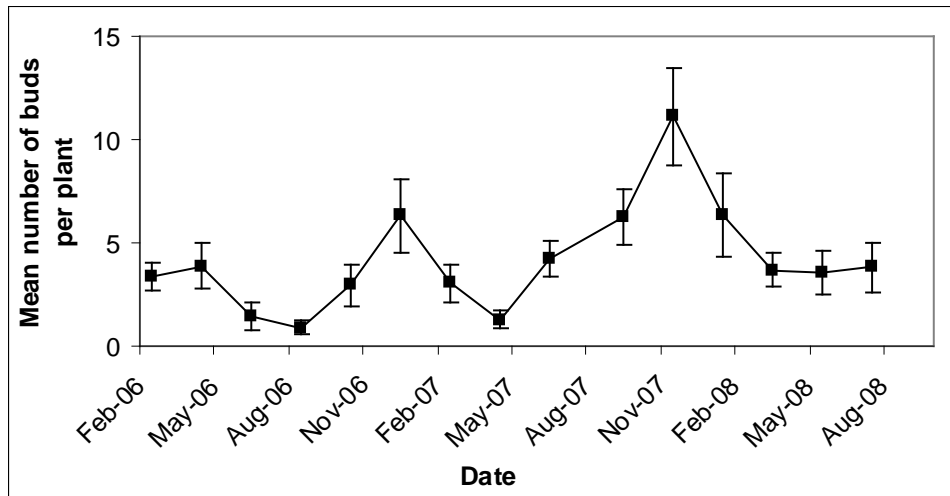


Figure 5. Mean number (\pm SD) of buds on *Cyrtandra giffardii* plants in `Öla`a Koa Unit, HAVO, 2006-2008.

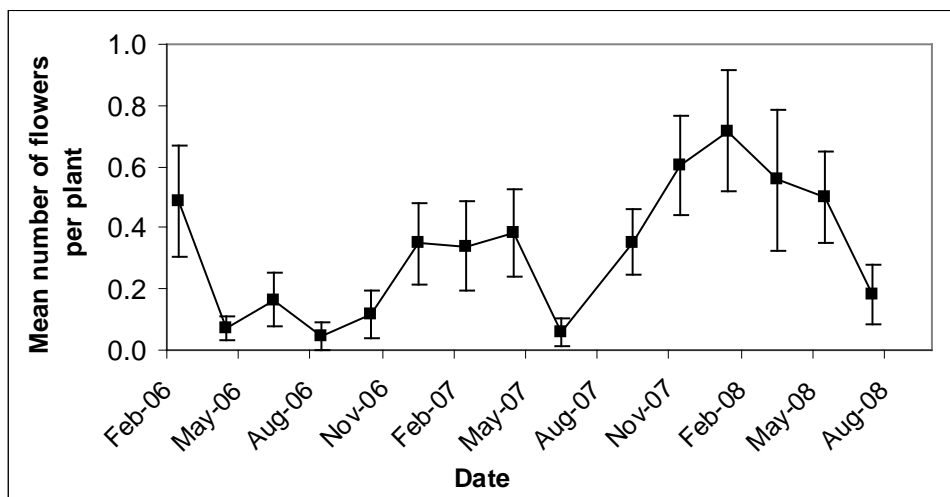


Figure 6. Mean number (\pm SD) of flowers on *Cyrtandra giffardii* plants in `Öla`a Koa Unit, HAVO, 2006-2008.

Immature, green fruit were observed on most plants (ca. 60-80%) in November and March, although a few plants displayed these structures at every monitoring period. Mature fruit were noted on fewer plants, but approximately 20% of plants displayed these structures between March and May of two sample years (Fig. 7). Peak production of immature fruit over three years was observed in November or February; between October 2007 and March 2008 there was a prolonged season of immature fruit production with the greatest mean number of fruit per plant observed during the study (11.1 ± 17.3 SD) (Fig. 8). Mature fruit were much less abundant, perhaps because many were dispersed in the intervals between monitoring periods. The highest mean numbers of mature fruit were noted in April 2006 (0.6 ± 1.7 SD), March 2008 (1.0 ± 2.4 SD), and May 2008 (1.0 ± 2.3 SD); very few mature fruit were detected in 2007 (Fig. 9).

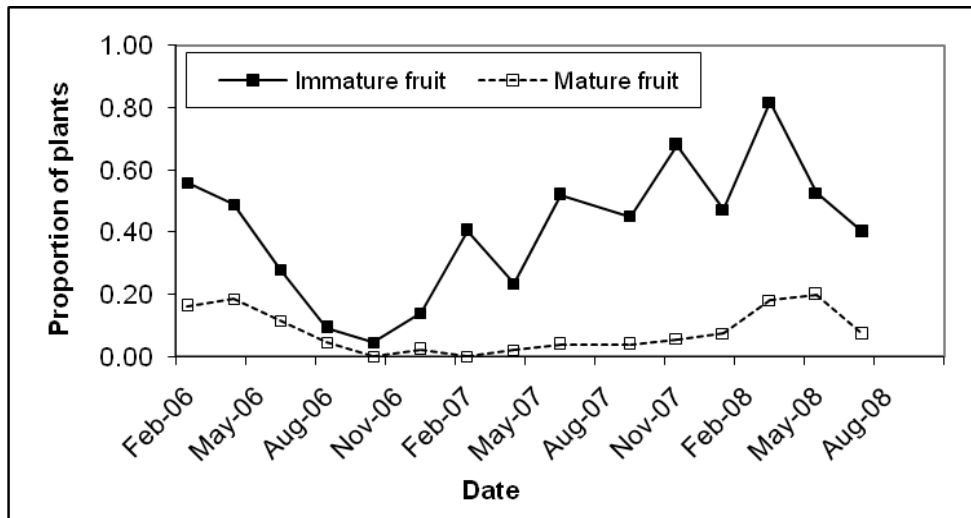


Figure 7. Proportion of *Cyrtandra giffardii* plants producing fruit in `Ōla`a Koa Unit, HAVO, 2006-2008.

Success of Flower and Fruit Production

Data from inflorescences that were tagged and monitored in 2007 and 2008 allowed us to determine the rate of successful transition from bud to either flower or fruit. Based on a large number of tagged buds ($n = 1238$), the detected bud to flower transition rate was only 4.8%; because flowers are ephemeral, it is likely that our two-month interval of monitoring was not short enough to accurately record this transition. Buds became immature fruit at a rate of

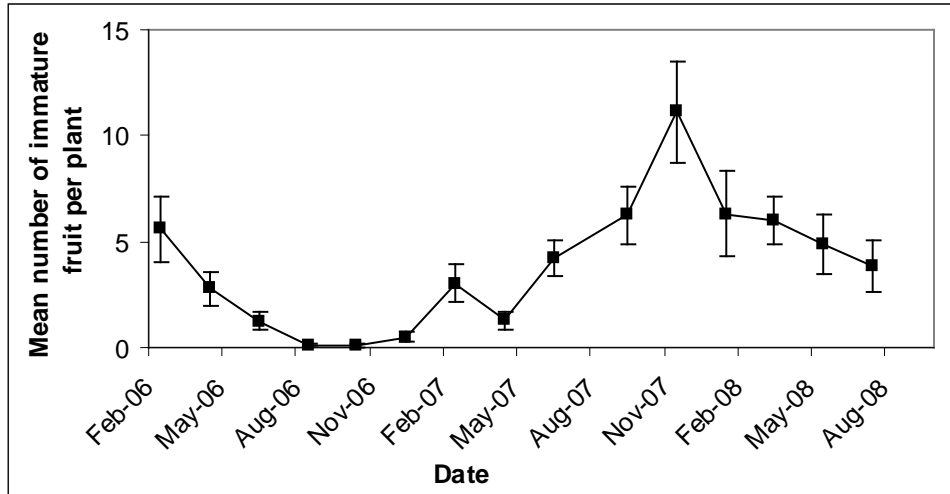


Figure 8. Mean number (\pm SD) of immature fruit on *Cyrtandra giffardii* plants in `Ōla`a Koa Unit, HAVO, 2006-2008.

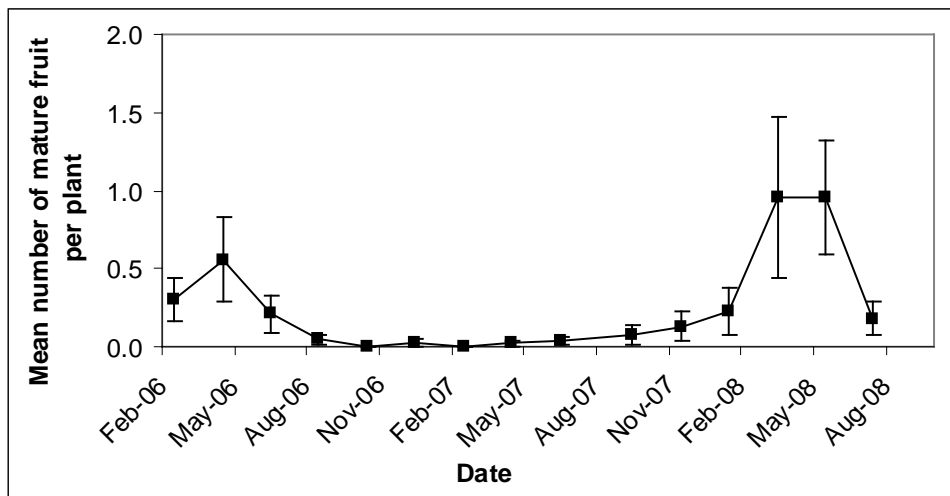


Figure 9. Mean number (\pm SD) of mature fruit on *Cyrtandra giffardii* plants in `Ōla`a Koa Unit, HAVO, 2006-2008.

26.0%, and this transition took on average 2.0 months (\pm 1.1 SD, n = 322). Open flowers successfully became immature fruit at a rate of 23.3%. Tagged immature fruit developed into mature fruit at a rate of 14.5%, and the average transition time from immature to mature fruit was 2.1 months (\pm 1.9 SD, n = 75).

Floral Visitor Observations

In 184.6 hours of video, no insects were recorded collecting floral resources from flowers of *C. giffardii*. A very small insect was observed repeatedly crawling around the exterior surface of the corolla, but never entered the corolla tube. Limitations of video resolution made it difficult to discern whether this insect was a micro-wasp (Hymenoptera) or fly (Diptera).

Cyrtandra giffardii and Hybrid Morphology

To quantify the morphology of sampled *C. giffardii* and likely hybrids we measured characters that should not overlap in the endangered *C. giffardii* and the more common *C. lysiosepala* found in the study site (except for pedicel length) (Table 1). Number of branches was highly variable with size of plant. Leaf shape and arrangement were consistently elliptic-ovovate and opposite; these characters were not useful for distinguishing *C. giffardii* plants. Pubescence was difficult to quantify, but most plants had scattered stiff hairs on the upper leaf surface and short hairs on the veins of the lower surface.

Table 1. Selected morphological characters of *Cyrtandra giffardii* and *C. lysiosepala* (extracted from Wagner *et al.* 1999).

Morphological Character	<i>Cyrtandra giffardii</i>	<i>Cyrtandra lysiosepala</i>
Branches	many	few
Leaf shape	elliptic-ovovate	elliptic
Leaf arrangement	opposite	opposite or whorled
Leaf length	6-12 cm	15-33 cm
Leaf petiole length	2-4 cm	4-10 cm
Peduncle length	25-35 mm	6-20 mm, stout
Pedicel length	15-30 mm, unequal	20-25 mm
Bract length	6-7 mm, linear	18-40 mm, foliaceous
Calyx length	3-9 mm, narrow	18-25 mm, foliaceous
Corolla tube length	12 mm	15-18 mm
Fruit (berry) length	1 cm, subglobose	1.6-1.8 cm, ellipsoid

Of the 61 plants measured, 18 were not fertile in 2008 or January 2009, and we have only leaf measurements. The other 43 plants had inflorescences at least once when visited. Nine plants had only 1 inflorescence measured, and 6 others had only 2 measurements during the sampling period. The remaining 28 plants had 3 to 19 inflorescences measured. Most

plants had at least 9 leaf measurements, 3 at each of three visits. In Table 2, the measurements for eight characters that fit those described for *C. giffardii* by Wagner *et al.* (1999) are highlighted in grey; in addition we accepted calyx and bract lengths to 1.0 cm. Fourteen plants had 5 or more measurements in the range of *C. giffardii*; only 1 plant was rejected because its fruit size was large. The 14 plants (with boxes around the plant identification number in Table 2) appear to represent *C. giffardii* and amount to 32.6% of the sampled plants fertile in 2008-2009. The other 29 fertile plants (67.4%) likely represent hybrids of *C. giffardii* and *C. lysiosepala*, although they have predominantly characters of the former species. All these fertile plants displayed leaf and inflorescence characteristics much closer to *C. giffardii* than to *C. lysiosepala*, which has large foliaceous bracts, long calyces with broad lobes, long corolla tubes, and large ellipsoid fruit (Wagner *et al.* 1999).

Density of *C. giffardii*, Hybrids, and *C. lysiosepala*

In three randomly-selected plots 20 x 20 m in size (400 m²) in `Ōla`a Koa Unit, there were no individuals that were unequivocally *C. giffardii*. The first plot had only 3 *C. lysiosepala* and 1 hybrid plant, and the other two plots had equal numbers of *C. lysiosepala* (7) and apparent hybrids between *C. lysiosepala* and *C. giffardii* (10). The extrapolated density of *C. lysiosepala* in this area of `Ōla`a was 158 plants/ha, and that of likely hybrid plants was 150 plants/ha.

Seeds and Seed Germination

Seed germination in the greenhouse. In the first germination trial for *Cyrtandra giffardii*, with 50 seeds sowed on wet filter paper, the tiny seeds began to germinate within 16 days of sowing and germination continued for 29 days. High germination rates of 60.0% and 72.0% were achieved by this method, but all seedlings died within two months of sowing (Table 1, Appendix II). The subsequent two trials in 2006 used seeds removed from pulp, counted, and surface sowed in replicate pots with a mixed medium. Only 1 of 6 pots had detectable germination; in this pot 12.0% of the sowed seeds germinated within five months. Out of 25 fruits with all seeds surface sowed in individual pots in 2007 and 2008, only 8 produced seedlings. The number of seeds per fruit was highly variable among those counted (13), ranging from 48 to 502. The mean of 233.3 seeds per fruit was used to calculate percentage

Table 2. *Cyrtandra giffardii* morphological character mean values (\pm SD) in cm. Grey indicates values within published range (except bract and calyx to 1.0 cm). Boxes indicate plants with 5 or more characters in range of *C. giffardii*.

Plant ID	Leaf Length	Petiole	Peduncle	Pedicel	Bract	Calyx	Corolla Tube	Ripe Berry	# Infl. Measured
2	8.7 \pm 0.8	1.2 \pm 0.2	-	-	-	-	-	-	0
3	12.5 \pm 2.3	2.0 \pm 0.9	2.0 \pm 0.1	1.9 \pm 0.1	1.0 \pm 0.1	1.0 \pm 0.1	1.2 \pm 0.1	-	3
5	10.4 \pm 1.1	1.2 \pm 0.2	-	-	-	-	-	-	0
6	14.4 \pm 1.4	1.9 \pm 0.4	2.9 \pm 0.8	2.6 \pm 0.8	1.0 \pm 0.2	1.3 \pm 0.2	1.0 \pm 0.0	-	8
7	15.2 \pm 1.5	2.4 \pm 0.7	2.7 \pm 0.1	3.3 \pm 0.0	1.7 \pm 0.0	1.5 \pm 0.0	1.3 \pm 0.0	-	2
9	17.8 \pm 3.1	4.9 \pm 2.5	3.3 \pm 0.4	3.4 \pm 0.3	1.3 \pm 0.3	4.5 \pm 5.9	1.5 \pm 0.1	-	13
10	12.1 \pm 2.3	2.6 \pm 1.5	3.0 \pm 0.5	3.2 \pm 0.5	1.2 \pm 0.2	1.2 \pm 0.1	1.2 \pm 0.0	-	5
11	13.4 \pm 3.2	3.0 \pm 2.2	3.2 \pm 0.3	3.4 \pm 0.6	1.5 \pm 0.1	1.4 \pm 0.1	-	-	2
12	15.5 \pm 3.9	2.8 \pm 1.3	3.2 \pm 0.7	2.5 \pm 0.3	1.2 \pm 0.3	1.0 \pm 0.2	1.1 \pm 0.1	-	8
13	15 \pm 1.6	2.8 \pm 1.3	-	-	-	-	-	-	0
14	15.2 \pm 1.5	2.0 \pm 0.2	-	-	-	-	-	-	0
15	12.6 \pm 1.7	2.4 \pm 0.3	1.8 \pm 0.2	1.7 \pm 0.1	1.1 \pm 0.1	1.2 \pm 0.0	1.2 \pm 0.1	-	3
16	14.4 \pm 2.1	2.3 \pm 0.6	1.8 \pm 0.3	2.2 \pm 0.4	1.1 \pm 0.4	1.4 \pm 0.1	1.5 \pm 0.1	-	4
17	13.6 \pm 1.2	2.7 \pm 0.5	-	-	-	-	-	-	0
18	14.8 \pm 2.7	2.2 \pm 0.4	-	-	-	-	-	-	0
20	14.4 \pm 2.7	2.2 \pm 0.5	4.0 \pm 0.5	3.8 \pm 0.5	1.0 \pm 0.3	1.2 \pm 0.1	1.0 \pm 0.1	1.3 \pm 0.3	15
21A	13.5 \pm 0.5	2.1 \pm 0.2	-	-	-	-	-	-	0
21B	7.5 \pm 0.5	0.9 \pm 0.1	1.9 \pm 0.1	1.5 \pm 0.1	0.5 \pm 0.0	0.5 \pm 0.0	0.8 \pm 0.0	-	2
22	12.4 \pm 1	2.3 \pm 0.9	2.9 \pm 0.1	3.3 \pm 0.4	0.8 \pm 0.1	1.0 \pm 0.2	-	1.4	3
23	14.3 \pm 2.2	1.7 \pm 0.5	2.9 \pm 0.3	2.5 \pm 0.3	1.0 \pm 0.2	1.1 \pm 0.2	1.3	1.4 \pm 0.3	14
24	8.7 \pm 3.1	1.3 \pm 0.6	1.5	1.6	-	0.9	1.1	-	1
25	11.4 \pm 2.2	1.3 \pm 0.3	3.1 \pm 0.3	2.5 \pm 0.3	0.5	0.5 \pm 0.0	-	-	2
26	7.9 \pm 1.5	1.3 \pm 0.3	2.5	1.6	1.1	1.0	0.9	-	1
27	10.4 \pm 1.8	1.6 \pm 0.3	3.0	3.0	0.7	0.8	-	1.0	1
28	12.1 \pm 1.8	1.7 \pm 0.4	2.1 \pm 0.3	2.7 \pm 0.4	1.0 \pm 0.3	1.0 \pm 0.3	1.3 \pm 0.1	1.3 \pm 0.2	16
29	12.5 \pm 1.7	2.3 \pm 0.7	1.9 \pm 0.1	2.6 \pm 0.4	0.7 \pm 0.1	0.8 \pm 0.2	-	-	10
30	14.9 \pm 1.8	2.2 \pm 0.4	2.7 \pm 0.3	2.7 \pm 0.0	0.9 \pm 0.1	0.9 \pm 0.1	1.1 \pm 0.1	-	3
31	15.7 \pm 3.1	3.4 \pm 1.0	1.5 \pm 0.2	2.1 \pm 0.3	1.3 \pm 0.3	1.3 \pm 0.2	1.5 \pm 0.1	-	8
32	16.1 \pm 3.3	2.8 \pm 0.6	3.5 \pm 0.5	2.5 \pm 0.5	1.5 \pm 0.3	1.3 \pm 0.3	1.0 \pm 0.0	1.1 \pm 0.1	5
33A	11.7 \pm 0.3	3.4 \pm 0.6	4.0 \pm 0.1	2.7 \pm 0.1	0.2	0.8 \pm 0.1	-	1.0 \pm 0.1	9
33B	10.8 \pm 2	2.0 \pm 0.8	2.6 \pm 0.1	2.2 \pm 0.2	0.5 \pm 0.3	0.7 \pm 0.0	0.8 \pm 0.0	1.0 \pm 0.0	3
34	13.8 \pm 1.7	2.6 \pm 0.7	1.6	2.0	0.9	1.1	1.4	-	1
35	16.4 \pm 2	2.8 \pm 1.3	-	-	-	-	-	-	0
36	11.2 \pm 2.2	1.8 \pm 0.6	-	-	-	-	-	-	0
37	21 \pm 5.1	4.4 \pm 2.3	4.0	3.7	0.7	0.8	1.0	-	1
38	20.2 \pm 4.7	3.9 \pm 2.3	2.7 \pm 1.0	3.3 \pm 0.2	0.8 \pm 0.4	1.1 \pm 0.2	0.8	-	3
39	19.1 \pm 4.6	3.3 \pm 1.3	3.2 \pm 0.2	3.3 \pm 2.8	0.6 \pm 0.1	1.2 \pm 0.1	1.3	-	2
40	14.5 \pm 3.5	2.5 \pm 1.0	3.9 \pm 0.3	2.9 \pm 0.3	0.9 \pm 0.1	0.7 \pm 0.1	1.1 \pm 0.1	0.9 \pm 0.1	19
41	10.3 \pm 2.2	1.7 \pm 0.7	-	-	-	-	-	-	0
42	17.7 \pm 1.9	3.3 \pm 0.7	1.8	2.6	1.7	1.4	-	-	1
43	9.4 \pm 0.7	1.5 \pm 0.1	-	-	-	-	-	-	0

Table 2
Continued

Plant ID	Leaf Length	Petiole	Peduncle	Pedicel	Bract	Calyx	Corolla tube	Ripe Berry	# Infl. Measured
44	12.6 ± 1.5	2.0 ± 0.4	3.5	2.1	0.9	0.8	0.4	-	1
45	13 ± 1	1.8 ± 0.5	-	-	-	-	-	-	0
46	12.6 ± 1.1	2.0 ± 0.5	-	-	-	-	-	-	0
47	12.8 ± 1.8	3.3 ± 1.2	1.9 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	1.5	-	3
48	8.4 ± 1.4	1.2 ± 0.3	-	-	-	-	-	-	0
49	12.7 ± 2.1	1.9 ± 0.4	2.4 ± 0.1	2.6 ± 0.6	1.5 ± 0.1	1.3 ± 0.1	-	-	2
50	16.1 ± 2.9	3.4 ± 1.5	2.3 ± 0.2	2.1 ± 0.3	1.1 ± 0.1	1.1 ± 0.1	-	1.2 ± 0.3	12
51	13.1 ± 1	1.5 ± 0.2	3.5 ± 1.0	3.2 ± 0.5	0.8 ± 0.2	0.9 ± 0.1	1.0 ± 0.0	-	3
52	12.6 ± 2.1	1.3 ± 0.4	3.2 ± 1.0	3.0 ± 0.6	0.6 ± 0.0	0.9 ± 0.2	1.1 ± 0.0	1.1 ± 0.2	5
53	13.8 ± 1.8	3.0 ± 1.2	-	-	-	-	-	-	0
54	14.3 ± 0.8	2.4 ± 0.5	2.6 ± 0.5	3.0 ± 0.5	1.1 ± 0.2	1.3 ± 0.0	1.3 ± 0.0	-	6
60	21.9 ± 4	4.2 ± 1.8	4.7 ± 1.0	3.1 ± 1.4	1.2 ± 0.4	1.3 ± 0.2	1.6 ± 0.5	1.4 ± 0.2	12
61	13.7 ± 2.6	2.5 ± 1.0	-	-	-	-	-	-	0
62	11 ± 2	2.2 ± 0.5	3.0	2.0	1.5	1.3	1.3	-	1
63	13.9 ± 2.3	2.5 ± 0.7	3.6 ± 0.7	2.9 ± 0.4	1.1 ± 0.2	1.0 ± 0.1	1.1 ± 0.1	-	13
64	10.5 ± 1.4	1.9 ± 0.1	1.4 ± 0.7	2.2 ± 0.5	0.9 ± 0.1	1.3 ± 0.2	1.4 ± 0.2	-	5
65	10.7 ± 1.4	1.8 ± 0.4	1.9 ± 0.1	2.6 ± 0.1	1.2 ± 0.0	1.3 ± 0.1	1.5	-	2
66	13.4 ± 1.6	1.8 ± 0.7	-	-	-	-	-	-	0
67	12.7 ± 2.1	1.9 ± 1.1	-	-	-	-	-	-	0
B	13 ± 1.8	2.1 ± 0.4	2.0	2.0	1.6	1.3	1.4	-	1

germination for those fruit that produced seedlings but did not have actual seeds per fruit counts. Germination rates for the 8 pots with seedlings ranged from 0.6 to 17.9% for those fruits with known numbers of seeds; rates were 0.4 to 19.3% for those fruits with an estimated number of seeds. When the date of first germination was recorded, seedlings appeared 9 to 10 months after sowing.

Cyrtandra tintinnabula, Ha`iwale

Size Class Structure and Mortality

The park's population of the endangered *C. tintinnabula* was found at an unusual geological feature in the unmanaged northeastern quarter of `Ōla`a (Fig. 10). When discovered, the size of the population was estimated to be about 50 individuals, but the limited mean height of surviving plants was 36.1 cm (± 33.8 SD) in 2007. Only 1 plant was fertile in 2007 with a few flower buds. The height class structure of all surviving plants was similar in

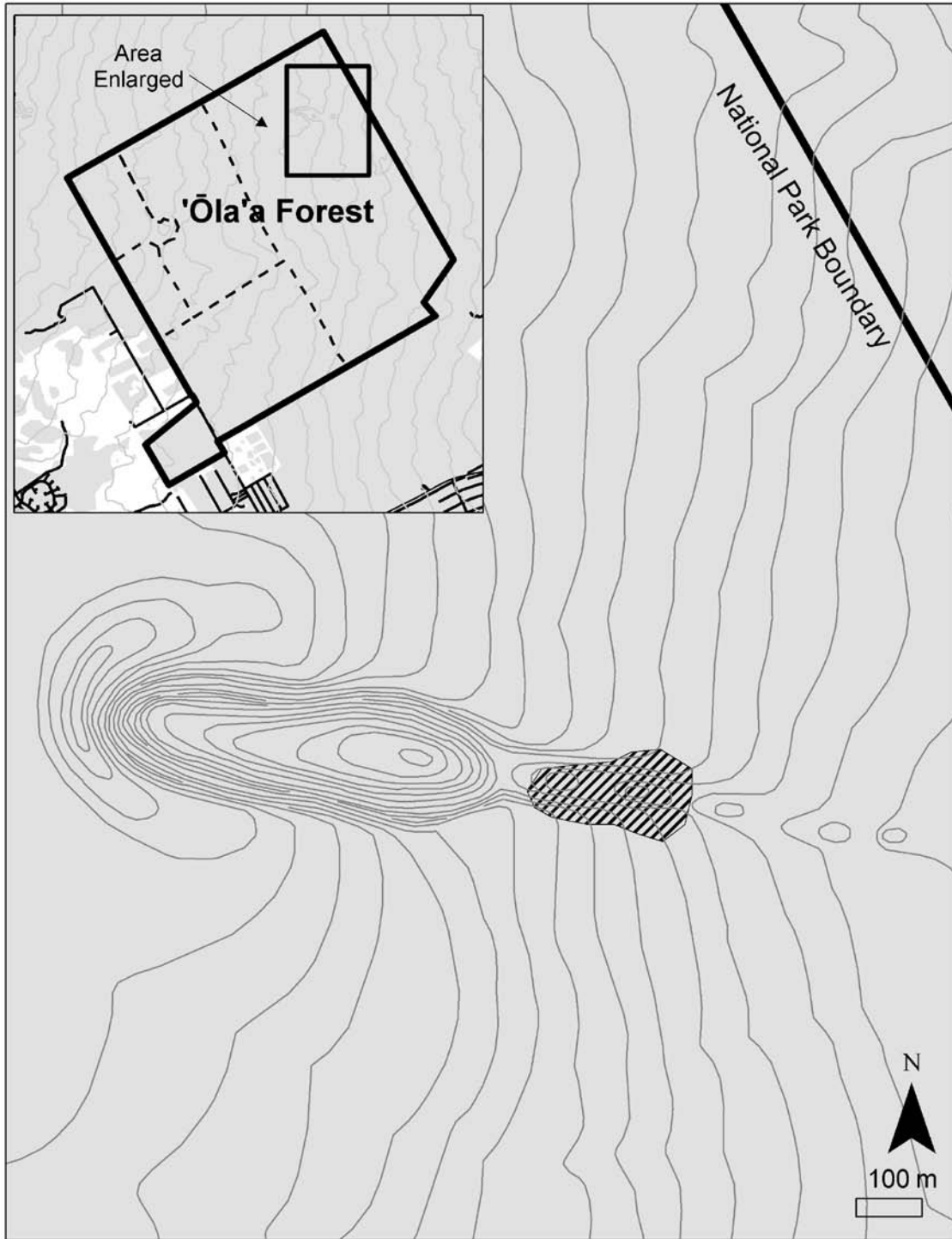


Figure 10. Location of *Cyrtandra tintinnabula* (striped area) in northeastern 'Ōla`a Forest, HAVO.

November 2006 and July 2007 with the best represented class that of plants 11-50 cm in height (Fig. 11). Young plants 1-10 cm tall were present at both monitoring periods; most of the plants in this group in 2006 survived and remained in the smallest size class in 2007.

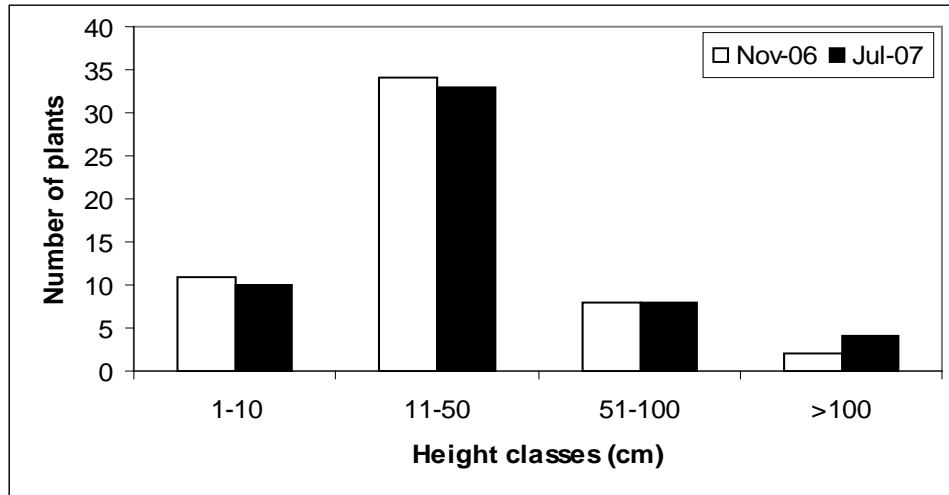


Figure 11. Height class distribution of 55 *Cyrtandra tintinnabula* plants in 2006-2007 at a site in the northeast quarter of `Ōla`a Forest, HAVO.

***Phyllostegia floribunda*, Mint (No Common Name)**

Growth and Mortality of Plantings

Phyllostegia floribunda is a natural component of the vegetation of `Ōla`a Forest that disappeared from a fenced unit in the 1990s. This species was introduced to Koa Unit, large tract in March 2003 and to small tract in June 2004 (Fig. 12) using cuttings collected at the adjacent Waiākea Forest Reserve and propagated at HAVO (Belfield, unpublished). Mortality of plantings was relatively low over three years of monitoring. Overall, 21.4% of 42 plants died during the study. A higher percentage of plants disappeared at the small tract planting site (27.8%) than at the large tract Koa Unit site (16.7%) (Fig. 13). Mortality was first noted on plants at the Koa Unit site more than a year after monitoring began, when plants were almost four years old. All plants selected for this study appeared to be in good condition in September 2005.

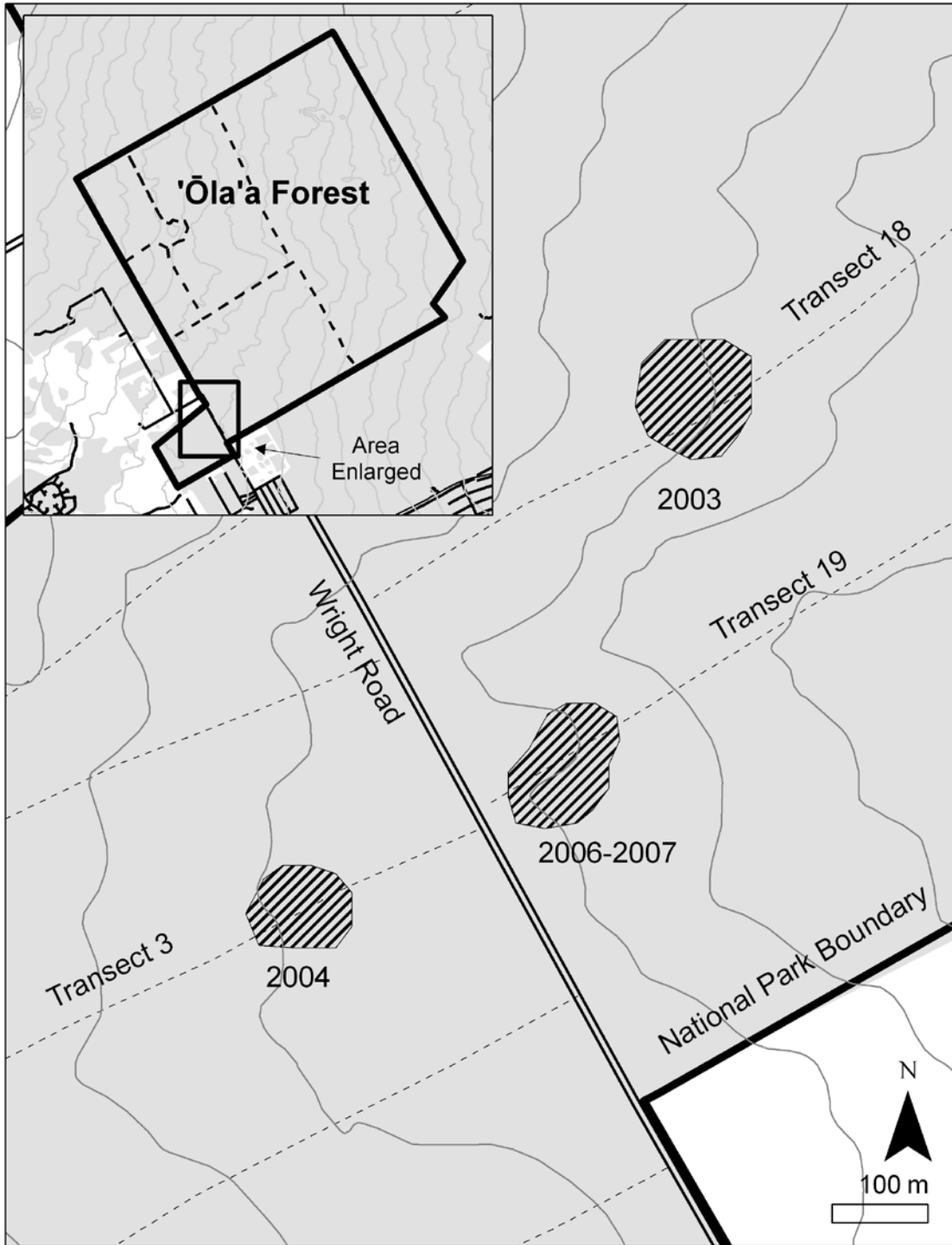


Figure 12. Location of *Phyllostegia floribunda* plantings (striped area) in 'Ōla'a Forest, HAVO.

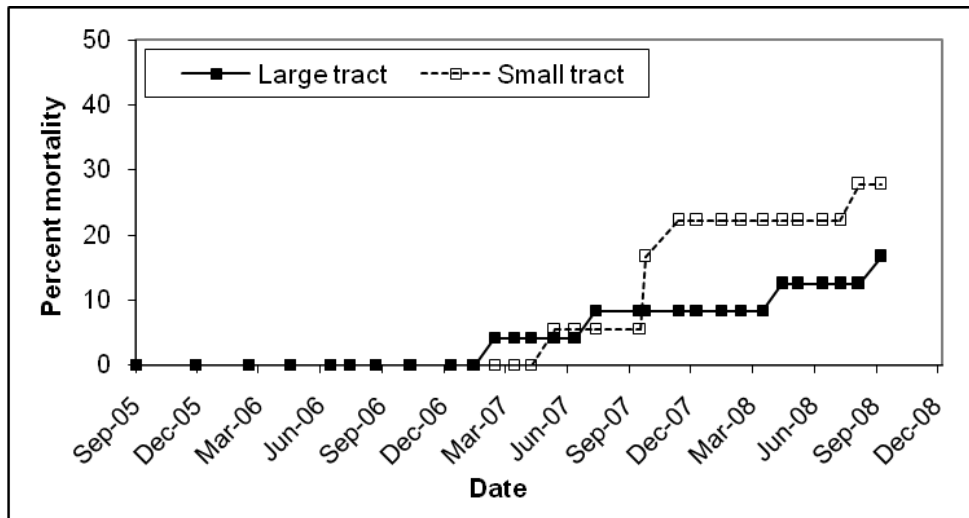


Figure 13. Mortality of planted *Phyllostegia floribunda* at two sites in `Ōla`a Forest, HAVO.

The younger plants of small tract were smaller than those of large tract in 2005, but heights at the two sites were similar after June 2006 through 2007 (Fig. 14). Mean height of *P. floribunda* plants fluctuated around 1 m in 2007, and small tract plants increased to a final mean height of 116.1 cm (± 52.2 SD, $n = 13$) in 2008. Periodic decreases in height were observed when older stems died and younger stems and resprouts were subsequently measured; by September 2008 large tract plants had decreased in mean height to 97.8 cm (± 35.1 SD, $n = 20$). By 2007, most plants had multiple stems. The mean number of stems in plants at the large tract site was significantly higher than that of the slightly younger plants at the small tract site ($t = 2.0$, $df = 25$, $p = 0.054$); at the end of the study the mean number of stems of large tract plants was 3.4 (± 0.6 SD, $n = 20$) and that of small tract plants was 1.9 (± 0.3 SD, $n = 13$).

Reproductive Phenology 2007-2008

Phenology of flowering and fruit production was monitored for 2 years; at the beginning of data collection *P. floribunda* plants had been in the ground for more than 3.5 years in the large tract Koa Unit (24 plants) and for 2.5 years in the small tract (18 plants). Almost all plants produced buds (81.0-92.9%) and flowers (76.2-83.3%) seasonally in both years with peaks between April and June or July (Fig. 15). The mean number of buds per plant was high

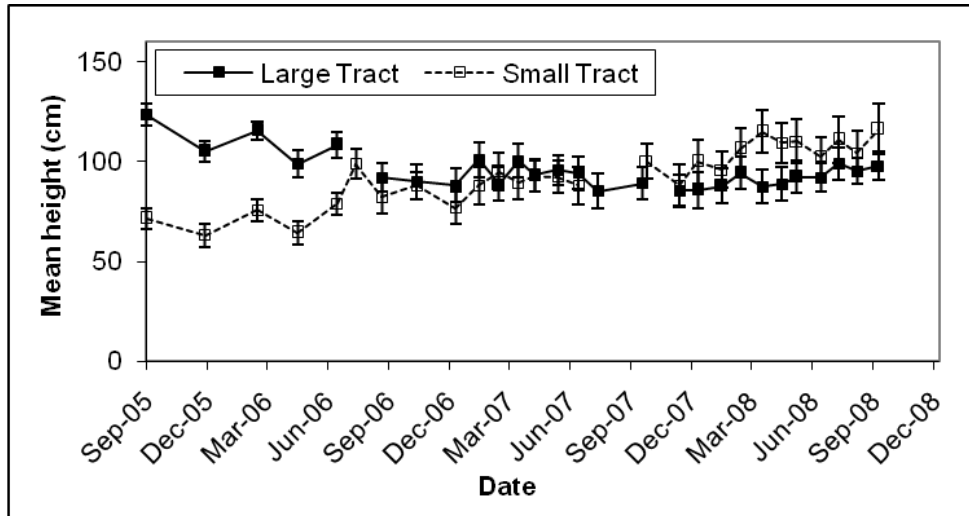


Figure 14. Mean heights (\pm SD) of planted *Phyllostegia floribunda* at two sites in `Öla`a Forest, HAVO.

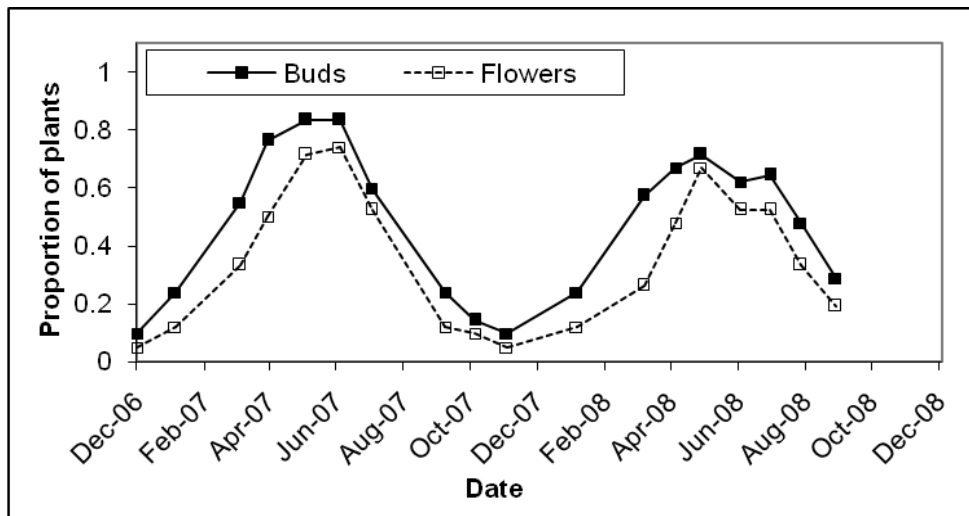


Figure 15. Proportion of planted *Phyllostegia floribunda* producing buds and flowers in `Öla`a Forest, HAVO, 2007-2008.

at the May to June peak in both years. At the seasonal peak in June 2007 the plant mean for buds was higher in small tract (127.3 ± 179.5 SD) than in large tract (37.0 ± 48.4 SD) where the peak was a month earlier with a mean bud count of $63.5 (\pm 92.5$ SD). At the bud

production peak in May 2008, the mean number of buds was similar at both sites with 53.9 (\pm 67.9 SD) buds per plant in small tract and 64.5 (\pm 78.8 SD) in large tract (Fig. 16).

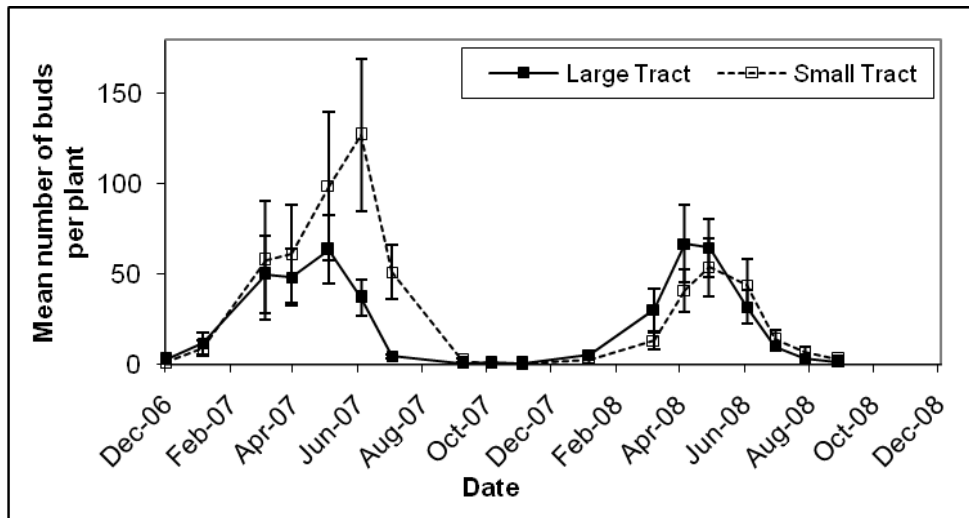


Figure 16. Mean number (\pm SD) of buds observed at *Phyllostegia floribunda* plantings at two sites in `Ōla`a Forest, HAVO, 2007-2008.

Flower production followed the same pattern as buds, with seasonal peaks in May or June. The mean number of flowers observed was much lower than the number of buds counted, but small tract plants again produced more flowers on average in 2007 than did plants in the large tract (Fig. 17). In the peak month of June 2007, the mean number of flowers counted per plant was 23.5 (\pm 36.6 SD) in the small tract and 6.3 (\pm 9.0 SD) at the large tract site. Peak flowering was slightly earlier in large tract in May 2007, when a mean of 10.8 (\pm 19.9 SD) flowers per plant was recorded.

The proportion of plants that produced immature fruit in 2007 was greatest in June when 88.1% of sampled plants had these developing fruit. The percentage of fruiting plants was somewhat lower in 2008 with a peak of 73.8% of plants with green fruit in July (Fig. 18). The proportion of plants observed with mature, black fruits was lower than that with green fruit in both years, and on most plants mature fruits were observed a month later than green fruit. In 2007, July had the most plants with mature fruit (54.8%), and in August 2008, 50% of sampled plants bore these fruit (Fig. 18).

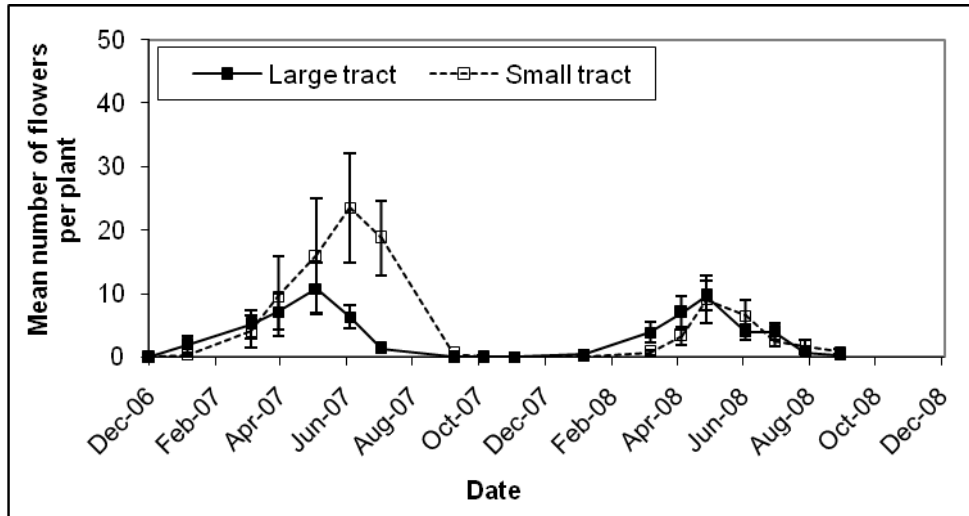


Figure 17. Mean number (\pm SD) of flowers observed at *Phyllostegia floribunda* plantings at two sites in `Ola`a Forest, HAVO, 2007-2008.

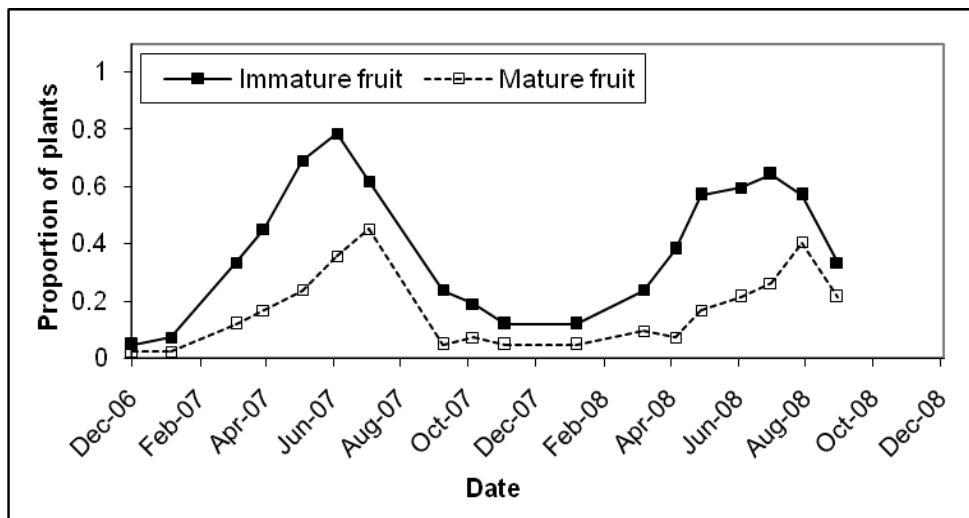


Figure 18. Proportion of planted *Phyllostegia floribunda* producing immature and mature fruit in `Ola`a Forest, HAVO, 2007-2008.

Plants sampled in the small tract had more immature fruits per plant in 2007 than did plants in the large tract, and the month of peak green fruit production was later. The greatest monthly mean for immature fruits recorded in small tract was 44.2 (\pm 66.3 SD) per plant in July 2007. Peak green fruit production in large tract was 24.8 (\pm 52.0 SD) per plant in May. Mean

immature fruit per plant were similar at the two study sites at the summer peak in 2008 (Fig. 19). The number of mature fruit detected was less than a quarter the number of immature fruit counted, and large tract plants had more mature fruit than those of small tract. The peak month for mature fruit production in 2007 was June when the mean number of black fruits per plant was 9.7 (\pm 20.8 SD) in large tract and 3.9 (\pm 12.8 SD) in small tract. In 2008, the greatest number of mature fruits per plant was recorded in May and August, when the large tract mean was 3.0 (\pm 7.9 SD) and 3.2 (\pm 6.1 SD) black fruits, respectively (Fig. 20).

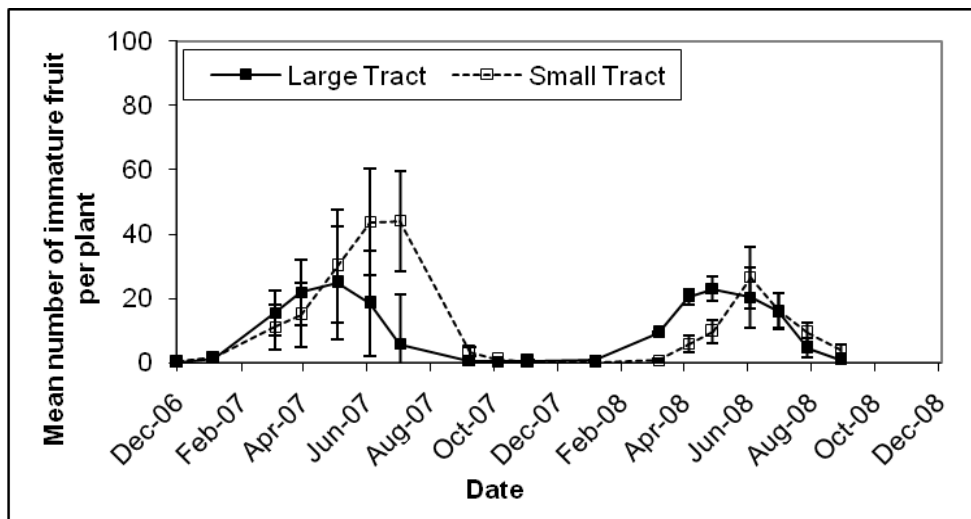


Figure 19. Mean number (\pm SD) of immature fruit observed at *Phyllostegia floribunda* plantings at two sites in `Ōla`a Forest, HAVO, 2007-2008.

Success of Fruit Production

During the season of peak flowering in June and July 2008, 51.5% of monitored flowers became immature, green fruit within two weeks. Within four to six weeks after flowers were tagged, 9.7% had developed into mature, black fruit. When expanded empty calyces that appeared to have held fruit were included with observed mature fruit, the percentage of flowers that successfully transitioned to mature fruit was 15.5%. The percentage of tagged immature fruit that successfully developed into mature fruit was 30.1%. When expanded empty calyces were added to the mature fruit present, the transition rate from immature fruit was 52.4%.

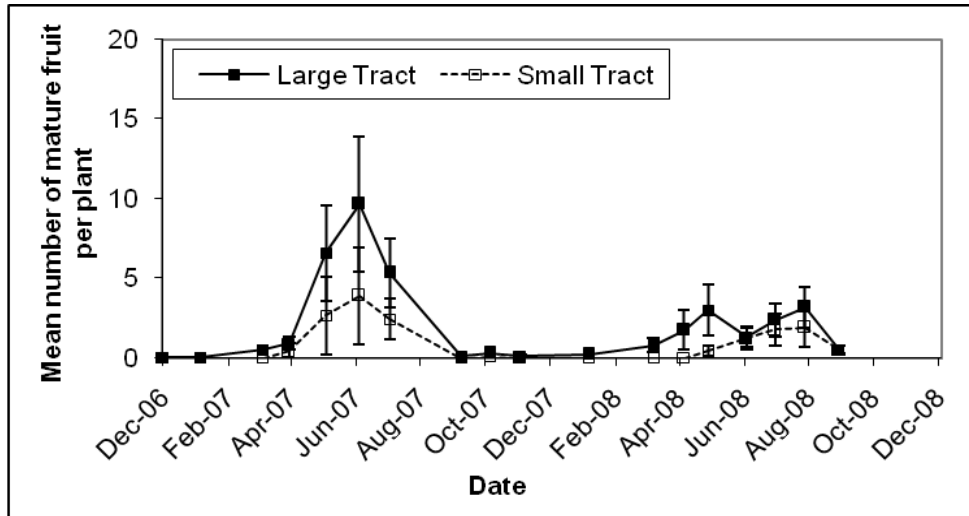


Figure 20. Mean number (\pm SD) of mature fruit observed at *Phyllostegia floribunda* plantings at two sites in `Ōla`a Forest, HAVO, 2007-2008.

Floral Visitor Observations

A total of 298 hours of video record was obtained, during which non-native honey bees (*Apis mellifera*), unknown fruit flies (Drosophilidae), and a native geometrid moth caterpillar (*Lophoplusia giffardi*) visited flowers of *P. floribunda*, resulting in 16 floral visits (Table 3). Honey bees were observed landing on the lower lip of a flower and inserting their heads down into the corolla contacting the anthers and stigma positioned on the upper lip of the corolla. Visits were infrequent with a mean visitation rate of 0.003 visits/flower/hr (\pm 0.019 SD, n = 14). Duration of a visit varied between 2 and 17 seconds, and the mean visit was 5.1 seconds (\pm 5.3 SD, n = 7). Fruit flies visited *P. floribunda* flowers on 7 different occasions with a mean visitation rate of 0.006 visits/flower/hr (\pm 0.026 SD, n = 14). Visits ranged from 28 to 1,424 seconds with a mean of 444.6 seconds (\pm 664.2 SD, n = 7). Fruit flies crawled around both the inner and outer surface of the corolla, but did not appear to be foraging for nectar or pollen. From the video recording, it was difficult to determine whether a flower's anthers or stigma were also incidentally contacted. The third floral visitor, an herbivorous geometrid moth caterpillar, was present on 2 occasions feeding on the foliage and flowers of *P. floribunda*.

Table 3. Visitation rates and duration of visits of insects on *Phyllostegia floribunda* flowers during timed observation intervals in `Ōla`a Forest, June to July 2007.

Floral Visitor	Status *	Total number of visits ¹	Mean visitation rate ² (SD) (n=14)	Mean visit duration in sec ³ (SD) (n = # of visits)
HYMENOPTERA				
Apidae				
<i>Apis mellifera</i>	pur	7	0.003 (0.019)	5.1 (5.3)
DIPTERA				
Drosophilidae				
Undetermined sp.	?	7	0.006 (0.026)	444.6 (664.2)
LEPIDOPTERA				
Geometridae				
<i>Lophoplusia giffardi</i>	end	2	n/a ⁴	n/a ⁴

* 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.

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 a flower based on individual visits independent of observation period.

⁴ N/a, not applicable. Insect herbivore destroyed flower.

Natural Seedlings

Over the course of two years of monitoring, 61 naturally-established seedlings were found adjacent to 6 planted *P. floribunda* at the large tract Koa Unit site. Twenty-four seedlings were noted in 2007, all at the base of a single *P. floribunda* plant. In 2008, an additional 37 seedlings were found; most of these were at the same plant that produced seedlings in 2007, but 12 appeared singly or in small groups at 5 additional *P. floribunda* plants. Most seedlings appeared in April or October, but groups of seedlings also appeared in December, January, and several summer months (Fig. 21).

Survival of seedlings was very poor, and at the end of monitoring in January 2009, only 8.2% of seedlings observed were still present. Almost half (42.6%) of seedlings died in less than a month and 60.7% were lost in their first 3 months. Six months after first appearance,

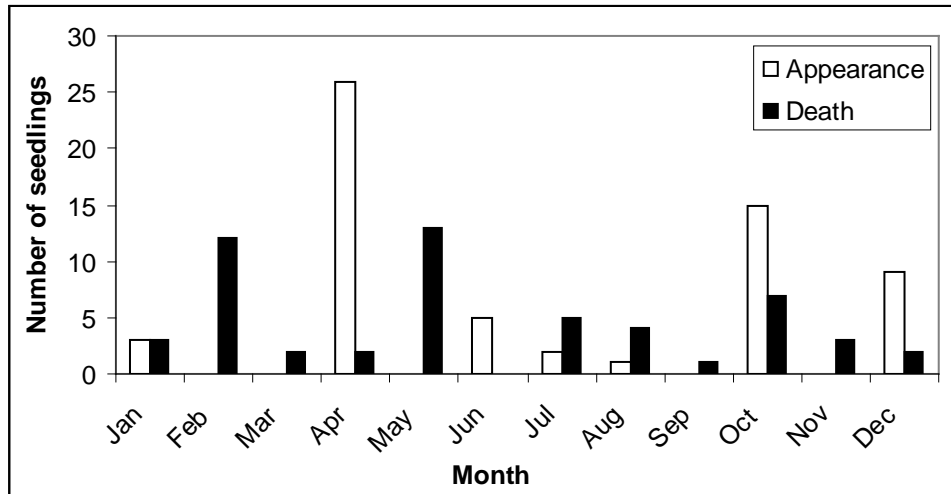


Figure 21. Time of year of *Phyllostegia floribunda* seedling appearance and loss at the large tract planting site in `Ōla`a Forest, HAVO.

the seedling mortality rate was 90.2%. The average life span of 61 seedlings was 3.6 months (± 3.6 SD). By the end of the study the mean height of surviving seedlings was 15.1 cm (± 15.0 SD).

Planted Seedlings in Sun and Shade 2006-2007

Survival and growth of seedlings. In the first planting (May 2006), seedlings grew rapidly for 11 months from <20 cm to a mean height of 93.2 cm (± 17.4 SD) in sun plots and 63.0 cm (± 17.1 SD) in shade plots (Fig. 22). Heights were greater in sun plots for the first 16 months, after which heights were similar in treatment and control plots. In their second year, heights of planted individuals began to decrease, and the mean fell to 41.9 cm (± 38.3 SD) in sun plots and 30.9 cm (± 24.3 SD) in shade plots after 27 months. Mortality of plants was observed first in shade plots after 7 months and was very low (5.0%) for 20 months. After 27 months, there was 35.0% mortality of *P. floribunda* in the shade plots and 25.0% mortality in the sun plots (Fig. 2.3); mortality of plants within sun and shade plots was not significantly different ($\chi^2 = 0.48$, $df = 1$, $p = 0.490$). When plots were revisited three years after the initial planting, only 3 *P. floribunda* plants remained alive in the 3 sun plots (85.7% mortality), and all plantings were dead in the shade plots.

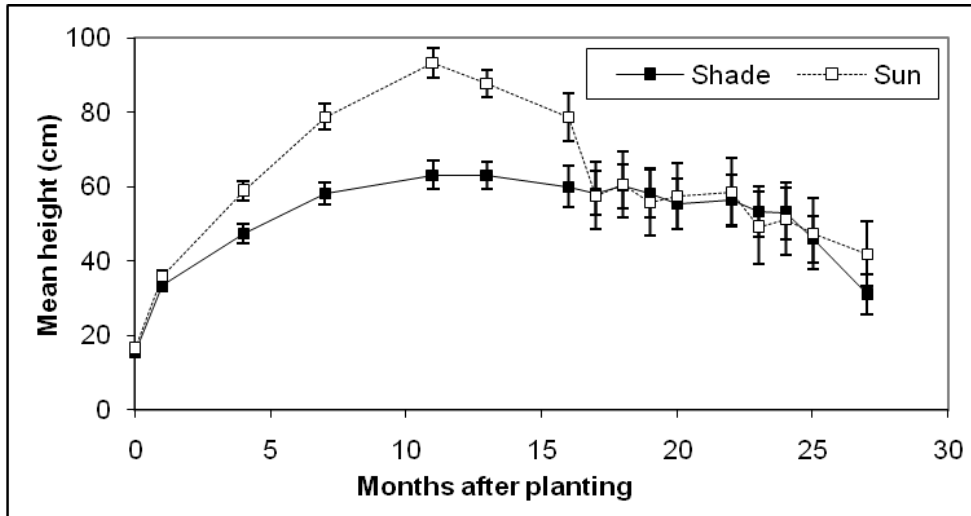


Figure 22. Mean heights (\pm SD) of *Phyllostegia floribunda* planted in May 2006 at sun and shade plots in `Ōla` a large tract, HAVO.

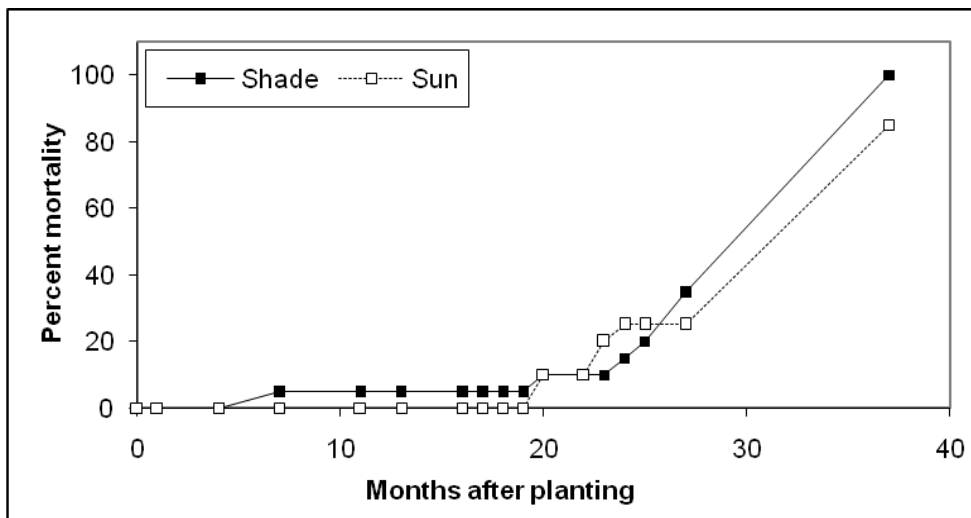


Figure 23. Mortality of *Phyllostegia floribunda* planted in May 2006 at sun and shade plots in `Ōla` a large tract, HAVO.

Growth was less rapid in the second planting, which took place in April 2007 (Fig. 24). Heights steadily increased in both sun and shade plots for 15 months, and by July 2008 the mean height of *P. floribunda* planted in 2007 was 70.5 cm (\pm 21.6 SD) in sun plots and 48.8 cm (\pm 15.8 SD) in shade plots. No mortality was observed for a year after planting, but after 15 months, 33.3% of shade seedlings had died and 22.2% of sun plot seedlings were lost

(Fig. 25). As with the first set of plants, the difference in seedling mortality in plots of the treatments was not significant ($\chi^2 = 1.02$, $df = 1$, $p = 0.310$). When plots were revisited in May 2009, 25 months after planting, all *P. floribunda* plants in the shade plots were dead and only 1 remained alive in a sun plot (94.4% mortality).

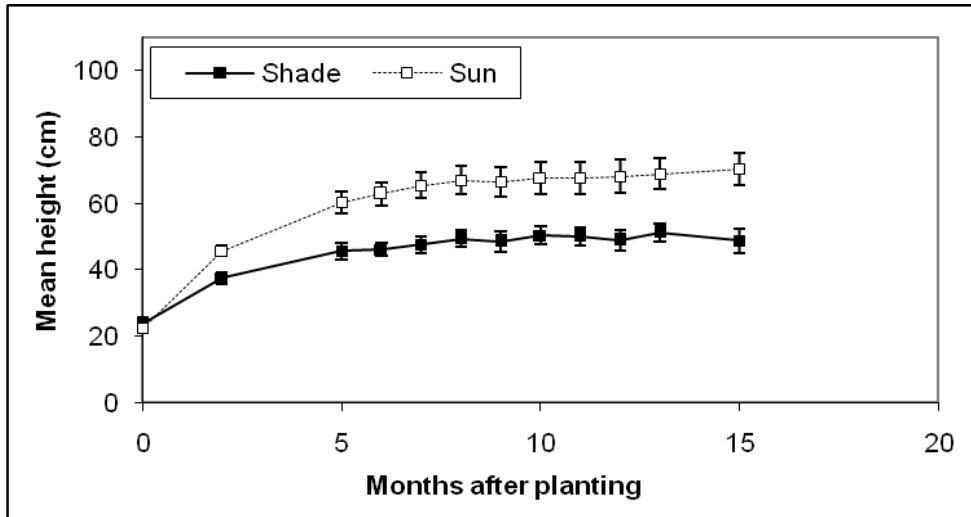


Figure 24. Mean heights (\pm SD) of *Phyllostegia floribunda* planted in April 2007 at sun and shade plots in `Öla` a large tract, HAVO.

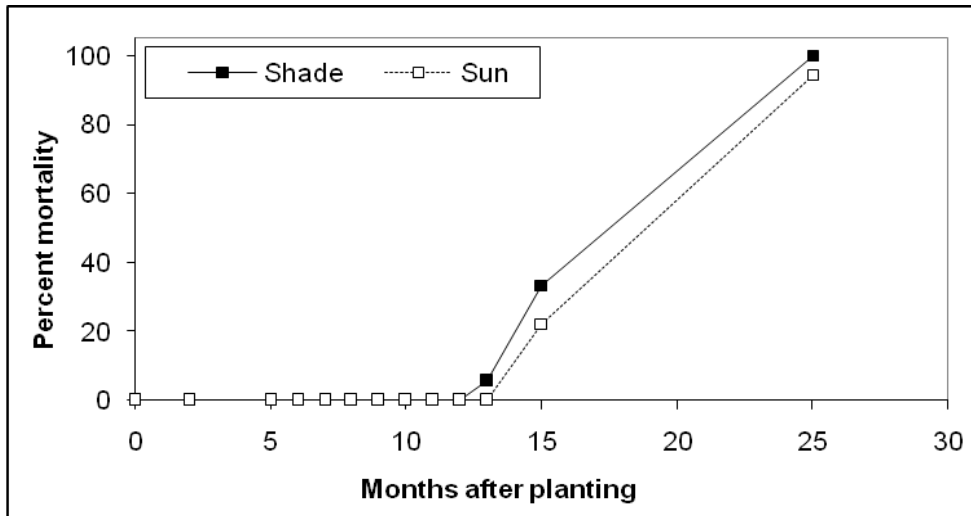


Figure 25. Mortality of *Phyllostegia floribunda* planted in April 2007 at sun and shade plots in `Öla` a large tract, HAVO.

Reproduction of seedlings. The first increment of seedlings reproduced after less than a year in the ground. Plants reproduced in both 2007 and 2008 and showed strong seasonality. At the first monitoring of reproduction in April 2007, peak bud production had already occurred, and the mean number of buds in sun plots was almost triple the plant mean in shade plots. In the second year of monitoring, buds were produced as early as January, bud numbers were relatively low, and there was no clear peak month during spring bud production (Fig. 26). Monthly mean number of buds was significantly higher in sun plots (18.0 ± 51.2 SD) than in shade plots (5.1 ± 18.8 SD), based on a repeated-measures analysis of variance ($F_{1, 31.1} = 16.0$, $p < 0.001$). Flower production peaked in June 2007 and April 2008, and the number of flowers produced in the plants' first year was much greater than in the 2nd year. As with buds, the number of flowers was approximately 3 times higher in sun plots than in shade plots during the first year (Fig. 27). The difference in the mean flower number in sun (3.2 ± 10.6 SD) and shade plants (0.9 ± 3.9 SD) was statistically significant ($F_{1, 122} = 8.0$, $p = 0.006$).

Immature fruit production appeared to be at or past its peak at the first monitoring of the first increment plants in April 2007, and in 2008 peak fruit production was in April (Fig. 28). As was observed for buds and flowers, the monthly means for immature fruit were much lower in 2008 than in 2007. Immature fruit production showed the same seasonal pattern as flowers, and sun plants (6.7 ± 21.2 SD) had higher overall mean numbers of immature, green fruit than

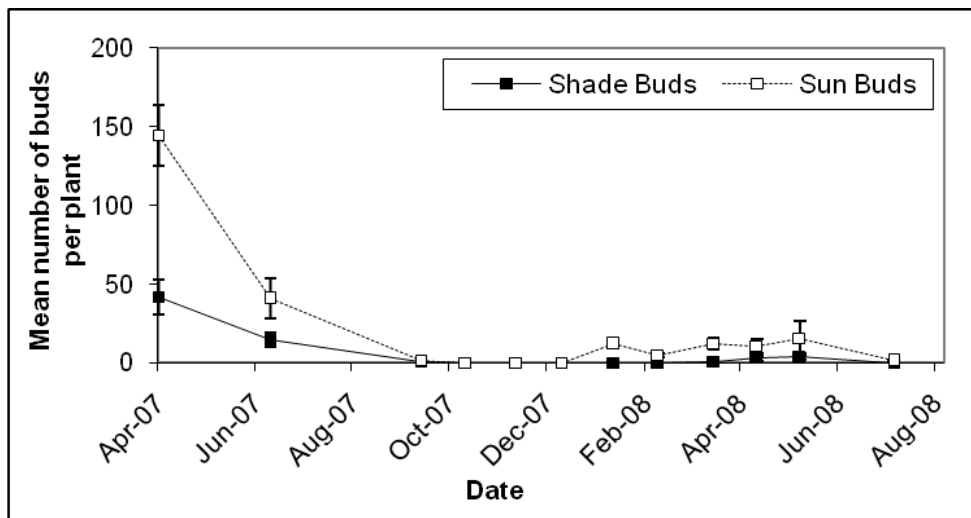


Figure 26. Mean number (\pm SD) of buds of *Phyllostegia floribunda* planted in May 2006 at sun and shade plots in `Öla`a large tract, HAVO.

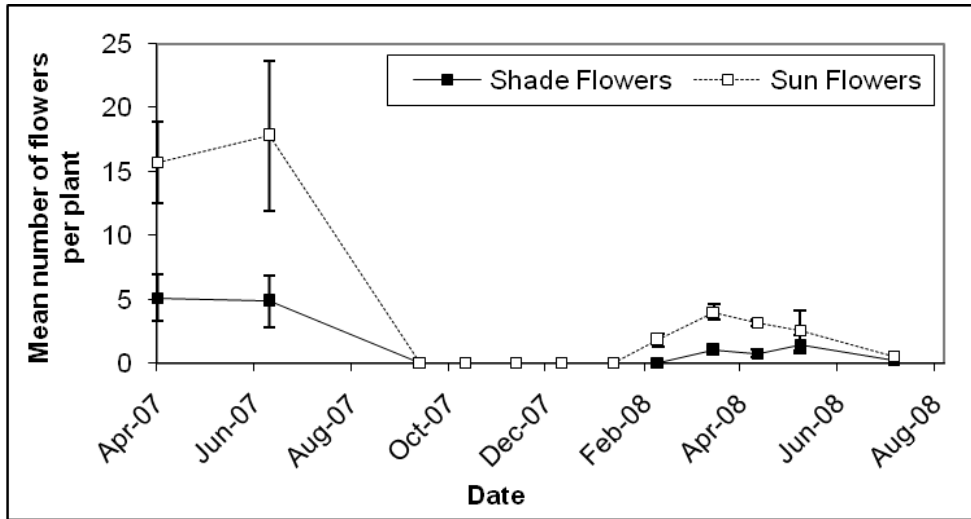


Figure 27. Mean number (\pm SD) of flowers on *Phyllostegia floribunda* planted in May 2006 at sun and shade plots in `Ōla`a large tract, HAVO.

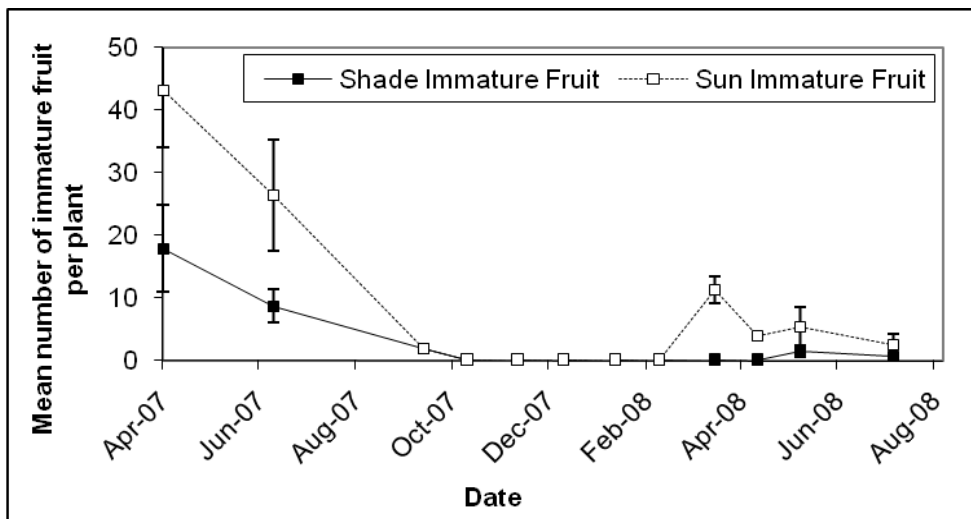


Figure 28. Mean number (\pm SD) of immature fruit on *Phyllostegia floribunda* planted in May 2006 at sun and shade plots in `Ōla`a large tract, HAVO.

did shade plants (2.3 ± 10.5 SD) throughout the monitoring period; this difference appeared to be significant ($F_{1, 57.9} = 5.6, p = 0.022$). Mature fruit production peaked in June 2007 and April 2008 and was limited to a few months (Fig. 29). The overall production of mature fruit per plant was similar in the two years, and sun plot plants produced more mature fruit on average (0.6 ± 3.0 SD) than did shade plants ($<0.1 \pm 0.4$ SD) throughout the monitoring period.

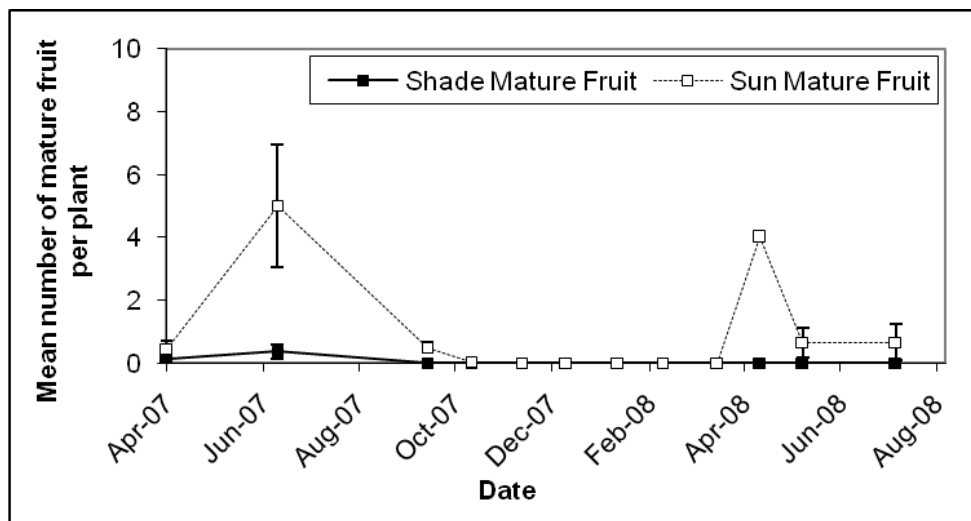


Figure 29. Mean number (\pm SD) of mature fruit on *Phyllostegia floribunda* planted in May 2006 at sun and shade plots in `Öla`a large tract, HAVO.

In the second increment of seedlings planted in sun and shade plots in 2007, there was almost no reproduction detected in the first year immediately after planting, and the seasonality of bud and flower production in 2008 was much like that of the first increment of plants with peaks near April (Fig. 30 and 31). Differences in the mean number of buds and flowers in sun and shade plots were less pronounced than for the first year of reproduction in the 2006 increment plantings. Production of immature fruit peaked in April and May 2008 with sun plants producing more fruit than shade plants (Fig. 32). Plants in sun plots produced a mean $8.8 (\pm 15.2$ SD) young fruit in April when shade plants averaged only $1.1 (\pm 1.8$ SD) fruit; shade plant immature fruit production peaked in May with a mean of $3.6 (\pm 6.6$ SD). Very few mature fruit were seen on second increment plants in sun plots, and these appeared primarily in May, when the mean of mature fruit was $1.2 (\pm 3.6$ SD). No mature fruit were noted on plants in shade plots.

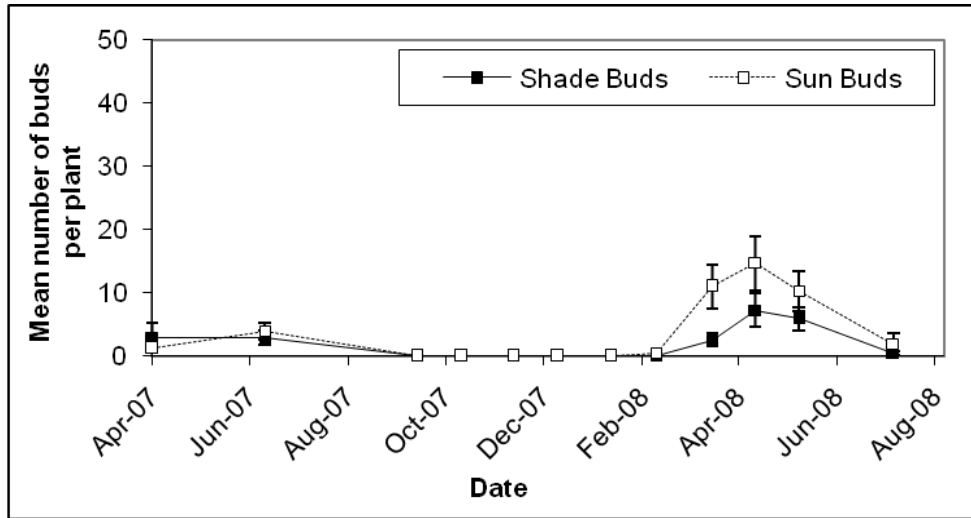


Figure 30. Mean number (\pm SD) of buds on *Phyllostegia floribunda* planted in April 2007 at sun and shade plots in `Ōla`a large tract, HAVO.

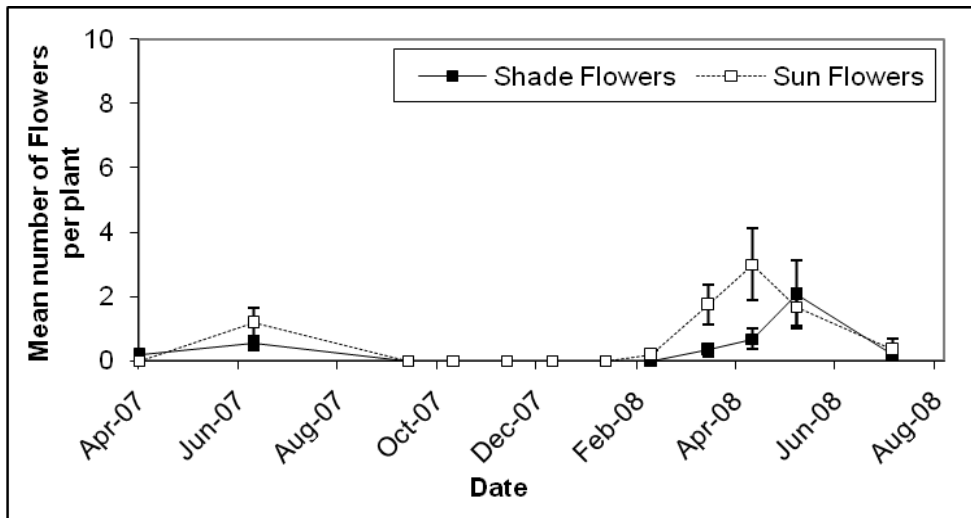


Figure 31. Mean number (\pm SD) of flowers on *Phyllostegia floribunda* planted in April 2007 at sun and shade plots in `Ōla`a large tract, HAVO.

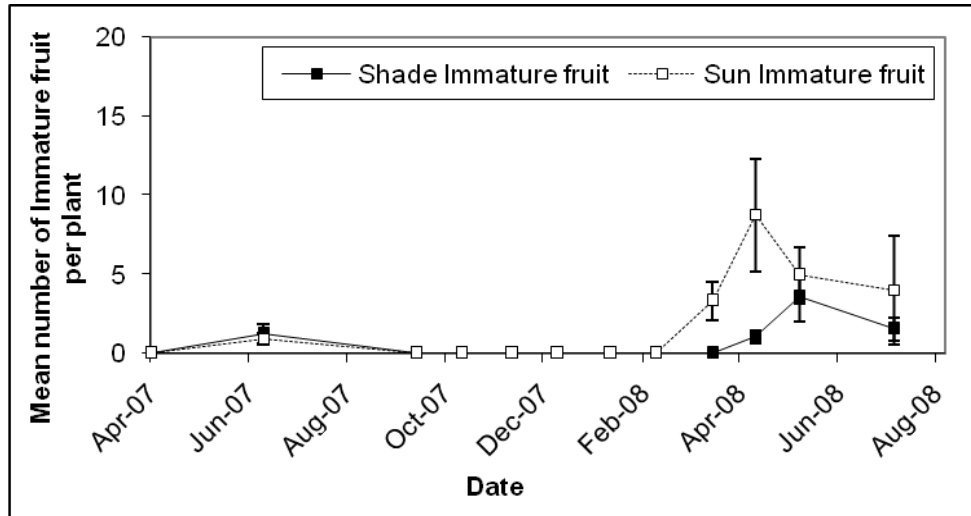


Figure 32. Mean number (\pm SD) of immature fruit on *Phyllostegia floribunda* planted in April 2007 at sun and shade plots in `Ōla`a large tract, HAVO.

Seeds and Seed Germination

Field seeding in sun and shade. Seeds were planted in June 2007 in each of the sunny plots with tree fern frond removal and each control plot in the shade. No seedling germination was observed in any plot during a year of monthly visits.

Soil seed bank. Seedlings first appeared three weeks after soil cores were planted in April 2009, but they could not immediately be identified to species. After four months, *P. floribunda* seedlings had appeared in 3 of 5 flats of soil cores. The mean number of *P. floribunda* seedlings per flat (each with five pooled cores) was 2.3 (\pm 3.2 SD). Other seedlings that germinated in the flats included the alien invasive shrub *Rubus ellipticus* (yellow Himalayan raspberry) and the native species *Solanum americanum* (pōpolo), *Perrottetia sandwicensis* (olomea), and *Pipturus albidus* (māmaki).

Seed germination in the greenhouse. Three germination trials were accomplished in July 2006, June 2008, and July 2008. Seedlings of *P. floribunda* first appeared in 2 of the trials 35-42 days after sowing; dates of first germination were not recorded for one trial. Germination rates were high in all trials; the overall mean germination rate in 2006 was 88.0% (Table 2, Appendix II). The mean of 5 pots with 25 seeds sown was 78.4%, and 2 flats with 50

seeds each had 100% germination. In 2008, the mean germination rate in each of two trials was 92.0% and 88.4%, and the second trial was still producing seedlings in March 2009 almost 8 months after sowing. Mortality of seedlings was recorded weekly in the 2008 greenhouse trials. In the trial sowed in June, mortality of germinated seedlings ranged from 22.5 to 24.0%. Excluding a flat that experienced a heavy downpour of rain from a broken glass panel on the roof during a rainfall event, mortality in the July 2008 germination trial ranged from 19.1 to 24.5%.

Seed viability test. The 18 *P. floribunda* seeds that were examined had been stored at 3 °C for one month, and none floated during the pre-test soak. Upon opening of the seed coat most seeds had endosperm and embryos of healthy appearance, although 4 of the seeds appeared partially decomposed and were not tested. Of the 14 seeds tested 3 were strongly positive (21.4%), 8 were positive (57.1%), and 3 were weakly positive (21.4%).

***Sicyos alba*, `Ānunu**

Four sites supporting the endangered vine *Sicyos alba* were visited at bimonthly or monthly intervals for more than two years (Fig. 33); these four sites had been previously found on or near transects surveyed for rare plants >15 years ago (Pratt and Abbott 1997). One other site that supported *S. alba* in 2000-2001 was revisited, and no live plants were found. The four monitoring sites have the only *S. alba* plants currently known within `Ōla`a Forest, but it is likely that other plants exist in unsurveyed portions of the forest.

It is uncertain how many plants were actually present at the monitored `Ōla`a sites, but at least five adult vines have been noted, and the actual number of individual plants is probably greater. The largest patch of vines (on transect 17) appeared to have at least two large vines rooted in the ground, but the life form of the species and its tendency to produce long branches that trail on the ground and loop from tree to tree made it difficult to discern individuals. The transect 18 site had 1 adult plant in 2005, which subsequently died. During the study this site supported natural seedlings of the former adult. A third site near transect 18 contained at least 2 adult plants and 1 young plant. The fourth site (on transect 19) had at least 1 adult plant and seedlings throughout the study.

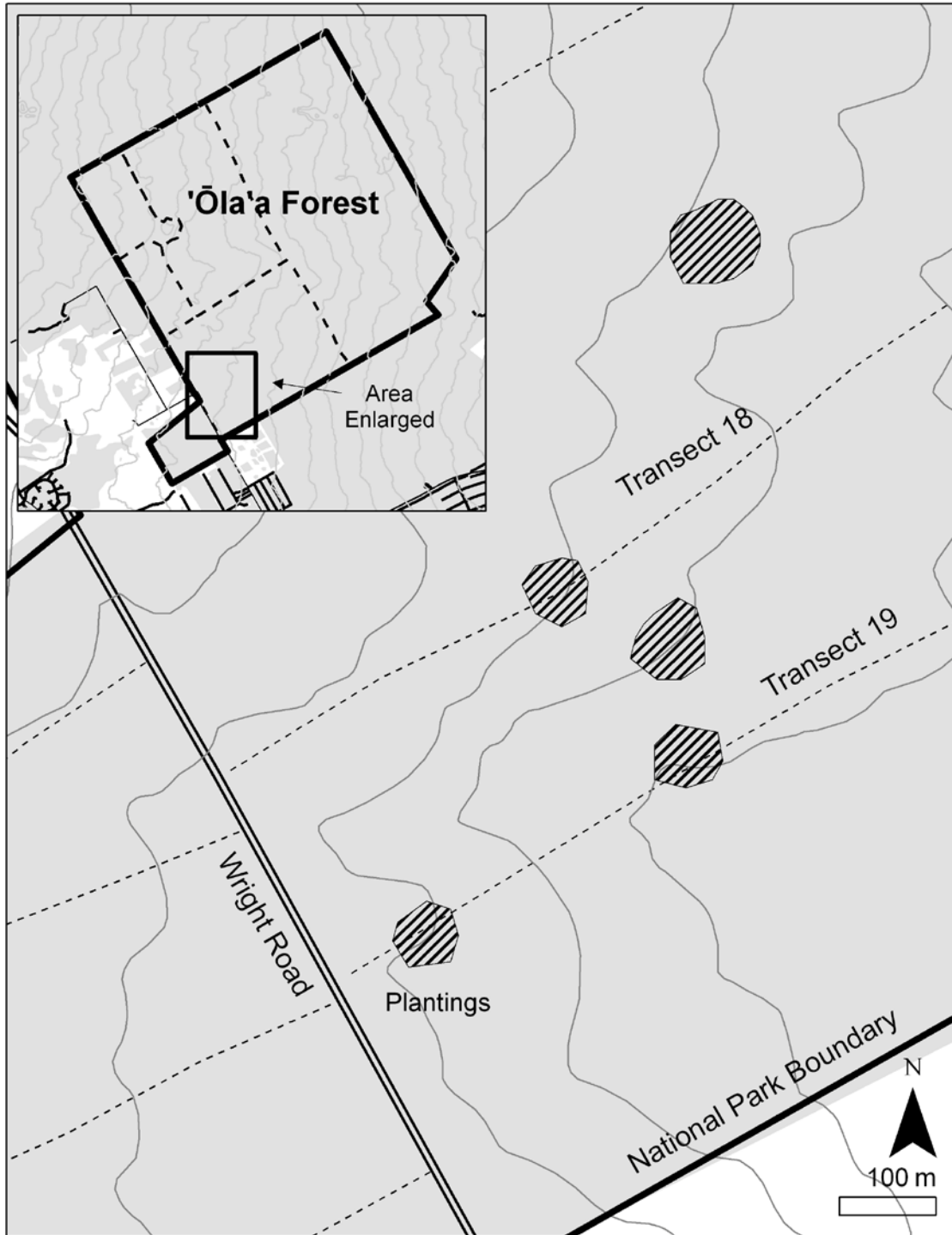


Figure 33. Location of *Sicyos alba* (striped areas) in 'Ōla'a Forest, HAVO.

Reproductive Phenology 2006-2008

Reproductive phenology was monitored at three of the four sites supporting *Sicyos alba*; at the fourth site, the adult vine had died prior to fall 2006 and only seedlings were present during the study. Flowering phenology appeared to be seasonal, but seasonal peaks were not consistent among years. Production of male inflorescences was prolonged with male flowers observed for nine months from September 2006 to May 2007 and for five months from May to September 2008. Peaks in male flower production were observed in March, September, and August, and few male flowers were present in June 2007 or December through March 2008 (Fig. 34).

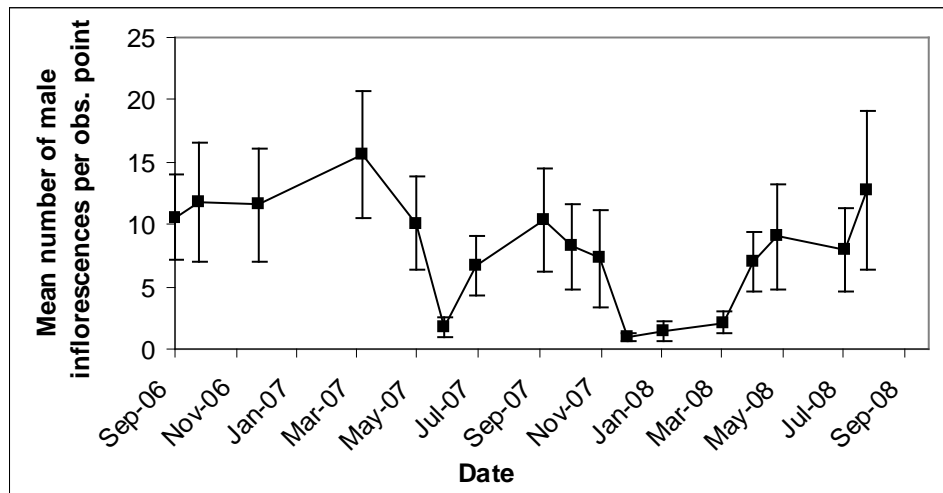


Figure 34. Mean number (\pm SD) of male inflorescences at 14 observation points of three sites supporting *Sicyos alba* in Ōla`a Forest, HAVO, 2006-2008.

Female inflorescences with buds and flowers were typically less abundant than were males at the observation points, and the seasonal pattern was not pronounced. Peak season for female flowers appeared to be May through September, and low points in female flower production were observed during months of November through January (Fig. 35). Inflorescences with developing small green fruit were consistently observed during July or August for three years (2006-2008) (Fig. 36). Large green fruit, essentially mature in size and shape, were persistent throughout the period of October to March or May during two years (Fig.

36). Peaks in inflorescences with mature fruit were also observed in March and September 2007 and August 2008. Low points for mature fruit occurred in July 2007 and March 2008.

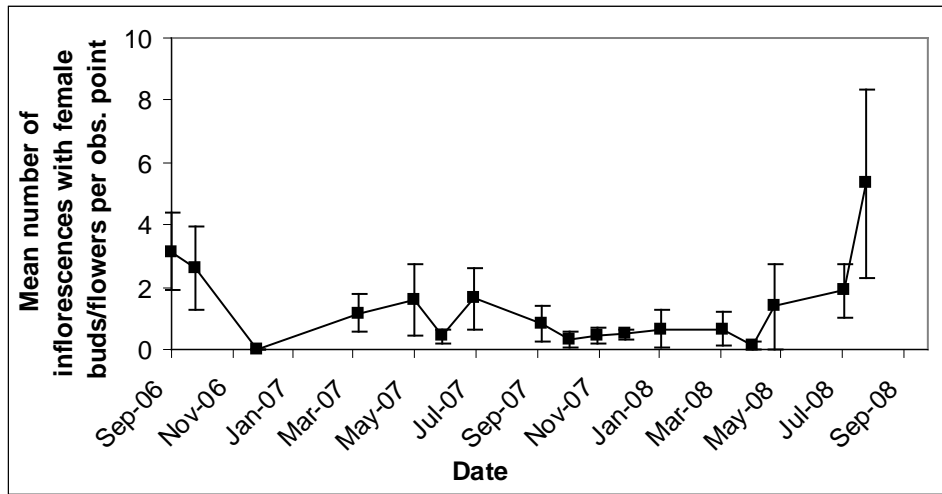


Figure 35. Mean number (\pm SD) of female inflorescences with buds and flowers at 14 observation points of three sites supporting *Sicyos alba* in Ōla`a Forest, HAVO, 2006-2008.

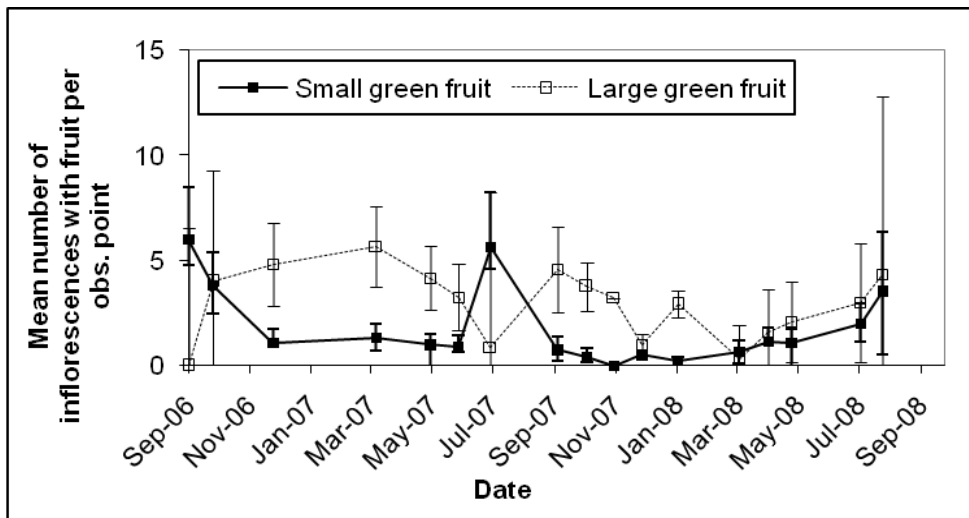


Figure 36. Mean number (\pm SD) of female inflorescences with small and large green fruit at 14 observation points of three sites supporting *Sicyos alba* in Ōla`a Forest, HAVO, 2006-2008.

Buds, Flowers, and Fruit on Female Inflorescences

Because the vine grew high into tree canopies, we were unable to reach a large sample of inflorescences, and we did not determine the success rate of female flowers to fruit. Our tagged inflorescences provided average numbers of structures per inflorescence. In a sample of 11 female inflorescences in an early stage of flowering, the mean number of buds was 2.4 (± 1.5 SD) and the mean number of flowers was 4.3 (± 2.2 SD). The mean number of small green fruit of 22 inflorescences was 4.6 (± 2.2 SD). Mature fruit averaged 1.3 (± 0.6 SD) on 3 older female inflorescences. Inflorescences were abundant on fertile sections of the vine; the mean number of inflorescences/m was 6.8 (± 2.6 SD) on 6 branches we tagged and measured.

Natural Seedlings

During the course of monitoring phenology of *Sicyos alba*, 33 natural seedlings were observed in total between June 2007 and May 2009 (Table 4). Most seedlings (66.7%) appeared between the months of March and June, although a few seedlings were noted in January, July, and August. Survival varied with the site; all seedlings noted at the largest group of vines (transect 17) had succumbed by the end of the study, but live seedlings remained at the other sites in May 2009 (groups of plants on and near transect 18 were pooled). Overall mortality of seedlings was 69.7%, and the lifespan of seedlings that were eventually lost averaged 3.0 months (± 2.5 SD, $n = 21$). The longest-lived surviving seedling persisted for 15 months (Table 4) and at the end of the study was climbing into the canopy of a tree fern. Most of the seedlings that died did not reach a height of 10 cm; growth of 7 surviving seedlings averaged 274.3 cm (± 178.4 SD). The maximum growth in height/length observed was 500 cm. Seedlings growing directly on transects appeared to be vulnerable to human damage; during the course of the study, several seedlings were observed with cut stems.

Planted Seedlings

Three seedlings that resulted from early germination trials were planted by HAVO Natural Resources Management Division staff in a plot also used to plant *P. floribunda*. Seedlings grew rapidly and achieved heights >2 m within a month of planting. Two of the plants continued to grow, climbing *Cibotium* tree ferns and *Metrosideros* tree trunks, until they

were at least 6 m tall or long. No reproduction was observed on any of the 3 plants. One plant died a year after planting and the others persisted for 16 months before dying.

Table 4. Appearance, growth, and mortality of natural *Sicyos alba* seedlings in `Ōla`a Koa Unit, HAVO, 2007-2009.

	TR 17	TR 18	TR 19
# Seedlings observed	18	10	5
Month of appearance	May-June	Jan, Mar-Aug	Jan, May, Aug
% Mortality	100%	30%	40%
Mean lifespan (mo)	2.6	3.0	11.0
Longest lifespan (mo)	7	15*	11
# Alive in May 2009	0	7	3
Mean growth in height (cm)	6.3	210.1	78.0

* Alive in May 2009

Seeds and Seed Germination

Seed predation experiment. Over the course of three months, fruit placed in bait stations in January turned dark and the flesh became soft and fell off. The first sign of predation was noted in March, when 1 seed in an open station was obviously predated and 2 others were missing from a second open station. After three months, predation was noted at all 3 open stations, and most seeds (93.3%) were either chewed by rats (leaving tooth marks) or missing (Table 3, Appendix II). Seeds in the inaccessible control boxes were intact; only 1 seed showed signs of damage from insects. The mean number of seeds predated or missing combined in open boxes was 9.3 (± 1.2 SD). The number of disturbed (missing and predated) seeds was significantly higher in open boxes compared with inaccessible controls ($\chi^2 = 48.7$, $df = 1$, $p = <0.001$).

Predated fruits were also noted on the ground beneath the six observation points at *S. alba* plants on transect 17. During a three-month period in spring 2008, most fruits counted on the ground at observation points showed signs of rat predation of the enclosed seeds. In March, 66.7% of 15 total seeds were predated; in April, 52.0% of 50 seeds on the ground

showed rat damage; and in May, 77.8% of the 9 seeds found on the ground were predated. We never observed predation to fruits still attached to the vines.

Soil seed bank. In 3 samplings of the soil seed bank at the most prolific site supporting *S. alba*, no whole seeds were detected. Only one rodent-predated fruit husk was recovered from one soil core in the January 2009 sample. At all sampling periods, fruit were observed on the plants in the prior monitoring period above the observation points where cores were taken.

Seed germination in the greenhouse. Germination rates were low in 2007. Clipping the tough inner fruit wall to the seed did not improve the germination rate; the trial sown in January with clipped fruit had an overall germination rate of 4.0%, while the trial with unclipped, cleaned fruit in July had a higher germination rate of 11.1%. The two trials in 2008 and 2009 exhibited no germination of *S. alba* seeds (Table 4, Appendix II). An early set of trials to determine the best potting medium was terminated prematurely by rodent predation of planted seeds. Subsequent germination trials were protected from rodents with wire screening.

Seed viability test. Twenty seeds of *Sicyos alba* were collected as mature green fruit. None floated while soaking. When opened, 5 of the fruit, despite being otherwise healthy in appearance, did not have developed seeds and were not tested. The remaining fruit had fully developed, healthy seeds, and all 15 (100%) had strongly positive staining.

Potential Limiting Factors of Three Rare Plant Species Investigated in this Study

Results of two years of monitoring indicated that adult mortality or lack of fruit production were not strong limiting factors for three rare plant species in `Ōla`a Forest (Table 5). No obvious pollinators were detected in floral visitor observations at *C. giffardii* plants, but two potential pollinators were noted at flowers of *P. floribunda*. Seed germination rates were low and variable for *C. giffardii* and *S. alba*, but seed viability was high for all species tested. A soil seed bank was detected for *P. floribunda* but not for *S. alba*, which was the only species for which rat seed predation was observed. Natural seedlings were observed at *P. floribunda* and *S. alba*, but mortality was high for both species.

Table 5. Potential limiting factors of three rare plant species in `Ōla`a Forest investigated in this study.

Factor	<i>Cyrtandra giffardii</i>	<i>Phyllostegia floribunda</i>	<i>Sicyos alba</i>
Stand structure	Stable	Planted	Unknown
Adult mortality	7.3%	21.4%	Unknown
Flower/fruit phenology	Annual	Annual	Annual/subannual
Fruit production	High	High	High
% Flower to green fruit	23.3%	51.5%	19.4%*
% Green fruit to mature	14.5%	52.4%	28.3%*
# Potential insect pollinators observed	0	2	n/a
Seed germination (greenhouse)	0-19.3%	88.0-92.0%	0-11.1%
Seed viability test positive & strong	n/a	78.6%	100%
Soil seed bank	n/a	Yes	No
Rodent predation of fresh seeds	n/a	n/a	93.3%
Natural seedlings observed	No	Yes	Yes
Natural seedling mortality	n/a	91.8%	69.7%

n/a Not tested or not observed.

* Calculated from average number of flowers and fruit per inflorescence.

DISCUSSION

Population Structure, Growth, and Mortality

Populations of the three naturally occurring rare plant species appeared to be stable in `Ōla`a Forest over the study period, and a large proportion of the restored population of *P. floribunda* persisted for more than three years. The size-distribution structure of the *C. giffardii* population had most plants in the middle height classes with few small or large plants. This structure indicates a stable or stationary population that is neither growing nor declining (Barbour *et al.* 1980). Small plants less than 0.5 m tall were uncommon, and seedlings identifiable to this species were not seen during the study (although several were noted on a visit to transect 16 plants in 2010). Based on greenhouse germination work, seedlings are very small when they appear and may remain very small for more than a year. Such seedlings would be very difficult to distinguish in `Ōla`a, which has a dense ground cover of ferns and other herbaceous plants. It is likely that most seedlings establish themselves terrestrially since few plants we monitored were epiphytic.

Sampled *C. giffardii* plants exhibited slow growth through the >2 years of the study, and this increase in size was reflected in the increase in number of plants of taller classes and the concomitant decrease in smaller height classes. This species of *Cyrtandra* is reported to have the greatest height range of Hawaiian species in the genus and may achieve a stature of 6 m (Wagner *et al.* 1999). The tallest small trees we measured were near 3 m in height; these were presumably old plants.

Nothing is known about the longevity of *C. giffardii* (U.S. Fish and Wildlife Service 1996), but it is presumed to be a long-lived perennial. Because plants previously mapped in the study site were not tagged, we cannot be certain that we sampled the same plants noted in the early 1990s, but most plants in the study were located at the same positions along transects as plants mapped in 1992-1994 (Pratt and Abbott 1997) and may have persisted for >15 years. The mortality rate we detected was relatively low, and losses were in all cases attributed to the natural cause of tree fall. Elsewhere in Malesia, members of the genus are reported to be sensitive to disturbance of the canopy and human activities such as logging (Cronk *et al.* 2005). In Hawai`i, disturbance from feral pigs has been reported as a reason for decline of populations of *C. giffardii* (U.S. Fish and Wildlife Service 1996). The removal of feral pigs from the fenced

`Ōla`a Koa Unit in the mid-1990s (Loh and Tunison 1999) may have reduced threats to this endangered species and contributed to the stability and persistence of the population in the protected area.

The population of *C. tintinnabula* in `Ōla`a could not be intensively monitored because of its remote locality. However, the structure of more than 50 plants sampled in 2006 and 2007 indicated a stable population with a large class of small to medium-sized plants. By contrast with *C. giffardii*, small plants <10 cm in height (presumably seedlings) were noted at the protected site on the steep walls of a crater, so this population may have the capacity for increase in the future. Mortality observed between 2006 and 2007 was higher than for plants of *C. giffardii*, but this reflects greater losses in the small size classes. Few plants or populations have been reported for this endangered species, and little is known of its life history (U.S. Fish and Wildlife Service 1996).

The *P. floribunda* population of `Ōla`a resulted from planting, and the plants at each of the sites we monitored were of the same age planted a year apart. Structurally, this was an even-aged stand of plants, which will approach a stable population only with the appearance of seedlings and persistence of young plants. By the end of the study, small tract plants were still increasing in height, while large tract plants were decreasing, perhaps indicating some decline in vigor in these slightly older plants. Heights may not be the best indicator of adult growth for this shrubby species. The number of stems per plant slowly but steadily increased at both sites throughout the study, indicating that new basal sprouts were being formed to replace those that died. This production of new basal shoots was also noted during the first year after planting *P. floribunda* in `Ōla`a (Belfield, unpublished).

Mortality rates of *P. floribunda* recorded during the current study did not take into account the death of plants during the first two years after planting. Belfield (unpublished) reported the loss of 6% of large tract plants in the first year after planting and an additional loss of 11% in the second year, for a mortality rate of 17% over a two-year period. This was very similar to the 16.7% mortality rate we observed for plants we monitored at the site in 2007-2008. When the early losses were added to those we observed, the mortality rate of large tract plants amounted to more than one-third of the plantings. Belfield did not report mortality rates of the small tract plants, which were approximately one year old when we started our study. We observed no losses for small tract plants during our first year of

monitoring, but plants began to decline and disappear in 2007, and by the end of 2008 the mortality rate of small tract plants was 27.8%. Based on the mortality results of Belfield and the current study combined, the two `Ōla`a sites seemed equally suitable to support a planted population of *P. floribunda*.

Because we were unable to distinguish individuals of *S. alba*, other than seedlings, we did not estimate the population size in `Ōla`a. Because adult plants have persisted at 3 of the 4 sites we monitored for more than 15 years (Pratt and Abbott 1997), it appeared that the groups of plants were capable of remaining in place, either by persistence of adult plants or by the recruitment of young plants. At the fourth site in our study that supported only seedlings, the largest of these juvenile plants appeared within a year of the loss of the adult, and some plants seen first as seedlings grew >5 m in length but did not attain large size or canopy cover approaching that of the other groups of vines. Based on our two years of monitoring, the species did not appear to be an annual as reported by Wagner *et al.* (1999) and St. John (1978b). We did not observe large vines dying back to the base on a yearly basis, although we did note the loss of masses of the vine in the canopy and subsequent new growth.

Patterns of Reproductive Phenology

Two of the species for which phenology was studied in `Ōla`a had annual patterns of flower and fruit production, and the third species showed inconsistent seasonality in production of reproductive structures. *Phyllostegia floribunda* had the most pronounced and consistent annual pattern for buds, flowers, and fruit over the two years of the study. Buds, flowers, immature fruit, and mature fruit were produced over a period of several months, but peak flowering and fruit production was in the late spring and early summer. Productivity was high with a mean of 24 to 44 young fruit formed on the 2003-2004 plants in a single month. Because fruit production was extended over several months, and mature fruit formed relatively quickly, the actual fruit production of a single plant was likely several times higher than the mean at any point in time. Each fruit has four seeds, so the average plant has the capacity to produce almost 100 seeds per month during the fruiting season. There was some evidence from the younger original planting in small tract and the seedlings we planted in 2006 that young plants were more productive of flowers and fruits in their first few years, although variable weather conditions may have also influenced reproductive success.

The pattern of reproduction of *Cyrtandra giffardii* was also basically annual but the period of bud, flower, and immature fruit production was extended over almost a full year with the brief seasonal low point of flower production between May and August and that of immature fruit from August to November. Mature fruit production was more obviously seasonal with few ripe berries observed in most months and peaks from February to June. The much higher mean number of immature fruit per plant compared with that of mature fruit suggests that not all young fruit are successful, although the two-month interval of monitoring may have been too long to adequately detect ripe berries.

The reproductive pattern of the vine *Sicyos alba*, the only monoecious species studied, was annual for female inflorescences with buds and flowers or young fruit, but subannual for male inflorescences with two peaks of male flowering in March and October 2007. Peaks in female inflorescence production were in the summer from July to September, but during the rest of the year few buds, flowers or young fruit were seen. The peak production of male flowers earlier than females may be a strategy to encourage out-crossing (Opler, Baker *et al.* 1980, Martén and Quesada 2001). This pattern of earlier male flowering and subannual male flower production was also noted in the related *S. macrophyllus* in mesic forest habitat of HAVO (Pratt *et al.* 2010). However, the summer production of female flowers was opposite to the pattern observed for *S. macrophyllus*.

Mature, large green fruit of *S. alba* seemed to be persistent for much of the year with the lowest numbers seen during the months of greatest female bud and flower production in 2007. This pattern was not consistent in the following year (2008) when mature fruit were present in each sampled month except March, at a time when few female buds and flowers were seen.

Annual patterns of reproductive phenology are typical in tropical tree species (van Schaik *et al.* 1993) and neotropical wet and dry forest shrubs (Opler, Frankie *et al.* 1980). In Hawai'i most native plant species for which phenology has been studied show annual patterns of reproductive phenology. Berlin *et al.* (2000) found annual patterns of flowering for all nine tree and shrub species studied in a rain forest on Maui. Continual fruit production, similar to that observed in our study for *C. giffardii* and *S. alba*, was noted for two tree species in the Maui study (*Cheirodendron trigynum*, ōlapa, and *Melicope clusiifolia*, alani). The dominant tree of most Hawaiian rain forests, *Metrosideros polymorpha*, was found to have a strongly seasonal

annual pattern of flowering in several studies (Carpenter and MacMillen 1973, Porter 1973, Berlin *et al.* 2000). Phenological studies of dominant trees in Hawaiian dry and mesic forests, such as *Sophora chrysophylla* (māmane) and *Acacia koa* (koa), have also demonstrated strongly seasonal annual patterns of flowering and fruiting (Lanner 1965, van Riper 1980, Pratt *et al.* 1997, Banko *et al.* 2002). The flowering phenology of 12 native tree species within and near HAVO was found to be annual (Lamoureux *et al.* 1981). Five rain forest species in the Mauna Loa transect and Kīlauea Forest had strongly seasonal annual patterns of flowering, but the timing of flowering peaks varied among the species from spring to summer to fall.

Pollination—Floral Visitor Observations

The pollinators of *Cyrtandra giffardii* remain unidentified. It is unknown whether the lack of observed pollinators was due to a low density of pollinator taxa within the *C. giffardii* population studied. Overall, native pollinator populations in Hawai`i have declined in abundance because of habitat alteration, competition with introduced pollinators for floral resources, and predation by introduced insects such as several species of predatory ants (e.g., *Pheidole megacephala*, or bigheaded ant, and *Linepithema humile*, or Argentine ant) and yellow-jacket wasps (*Vespula pensylvanica*) (Cole *et al.* 1992, Gambino 1992, Magnacca 2007). Although insects were not observed visiting the interior of flowers of *C. giffardii*, fruit production was still relatively high. Further studies may be needed to determine if this species is self-compatible and capable of self-fertilization. A related species on O`ahu, *Cyrtandra grandiflora*, was found to be self-compatible, but did not set fruit when pollinators were excluded from flowers (Roelofs 1979). *Cyrtandra* species of Fiji also had no fruit set when bagged to exclude cross-pollination (Gillett 1967). Roelofs (1979) found no native pollinators on *C. grandiflora* but observed the alien sphinx moth *Macroglossum* sp. visiting flowers and taking nectar. She speculated that a crawling insect within the corolla tube would be most likely to cause self-pollination of *Cyrtandra* flowers.

The apparent hybridization in the population between *C. giffardii* and *C. lysiosepala* indicated that a pollinator must be present as a pollen vector between the two species. Flowers that rely on moth pollination are often white (Kevan and Baker 1983). Both *C. giffardii* and *C. lysiosepala* have flowers pigmented white (as do all Hawaiian *Cyrtandra*), which suggests moths as a probable pollen vector. Those *Cyrtandra* species that have been examined for ultraviolet

floral patterns have been found to lack markings, implying that their pollinating insects may not be using these patterns as floral cues (Jones *et al.* 1999).

A potential pollinator of *P. floribunda* was observed, but it was not a native species. Flowers of *Phyllostegia floribunda* were visited by a limited number of insect taxa with extremely low visitation rates. In 298 hours of observation, floral visits by a honey bee (*Apis mellifera*), fruit flies (Drosophilidae), and a geometrid caterpillar (*Lophoplusia giffardi*) made up only 16 total visits. The only documented observations of an insect harvesting a floral resource and potentially affecting pollination were those of honey bees. Honey bees collect both nectar and pollen from a wide variety of native and non-native plant species in Hawai'i (Arita *et al.* 1989). The fruit flies did not appear to forage for either nectar or pollen, and instead they may have been utilizing flowers to oviposit eggs. The geometrid caterpillar fed on the leaves and flowers of *P. floribunda*, which documented a new host plant species for *L. giffardi*. Typically, geometrid caterpillars feed on leaves, but they may also eat flowers, pollen, fruits, and lichens (Hausmann 2001).

Success of Fruit Production

Two of the species for which reproduction was studied exhibited relatively high rates of successful fruit production: *C. giffardii* and *P. floribunda*. As for the third species we studied, *Sicyos alba*, it was not possible to tag and revisit a sample of inflorescences to determine fruit set. However, observations of the mean number of female buds, flowers, and fruit per inflorescence on this species indicated that 19.4% of flowers and buds formed mature fruits. This was comparable with the fruit set observed in open-pollinated flowers of the mesic species *S. macrophyllus* (Pratt *et al.* 2010).

Cyrtandra giffardii buds successfully developed into immature fruit at a rate of 26.0% and the rate of transition from flower to immature fruit was similar at 23.3%. The rate of development of mature fruit from immature fruit was lower at 14.5%, but this likely does not account for fruit that ripened and dispersed in the two-month monitoring interval. We have no comparable data from other studies on this endangered species, but Roelofs (1979) studied the reproductive biology of the related *C. grandiflora* on O`ahu and reported fruit set of open-pollinated flowers ranging from 32 to 39% over a period of two years.

Phyllostegia floribunda had the greatest rate of successful fruit production of the three species studied. Flowers successfully transitioned to immature fruit at a rate of 51.5%, even though the rate of formation of mature or dispersed fruit from flowers was a lower 15.5%. Because *P. floribunda* developed quickly, it is likely that our two-week interval of monitoring was not short enough to detect all mature fruit before they were dispersed and the inflorescence deteriorated. The 52.4% development of mature fruit from immature fruit is more evidence of the high reproductive success rate for this species. Fruit production did not seem to be limited in this species, and rates this high indicate that successful pollination is occurring. Rates of fruit production that we observed in *P. floribunda* are comparable to the high rates of fruit set (55%) in *Metrosideros polymorpha*, the most successful tree species of Hawaiian forests (Carpenter 1976). Fruit set of the rare *P. floribunda* is higher than the 15% rate observed for *Acacia koa*, koa (Lanner 1965) or the 17% successful pod production reported for *Sophora chrysophylla*, māmane (van Riper 1980); both trees are widespread species and community dominants in some Hawaiian forests.

Seed Germination, Soil Seed Banks, and Seedlings

Seed germination rates of the wet forest species we studied were high for *P. floribunda* and low and highly variable for *C. giffardii* and *S. alba*. Seeds of *P. floribunda* had greenhouse germination rates >88% in both years of trials. All of our trials were carried out in either June or July when fresh seeds were available, so we do not know if germination rates would be reduced in the cooler winter months. Plant propagators at HAVO have reported high germination rates for other species of *Phyllostegia* grown for rain forest restoration projects (S. McDaniel, pers. comm.). The time from sowing to germination we observed for the species is comparable to the two weeks to three months reported for the genus (Lilleeng-Rosenberger 2005). Some species of *Phyllostegia* were reported to have physiological dormancy (Baskin *et al.* 2004). While most of the seeds in our germination trials germinated within three to five months after sowing, germination continued for as long as eight months in one trial; this prolonged germination period may be related to dormancy in seeds of the species.

The single soil seed bank sampling we carried out indicated that *P. floribunda* seeds were capable of germinating after eight months in the soil. The last fruit production we noted was in August 2008, and because of the seasonality displayed by the species it is unlikely that additional seeds were added to the soil bank we sampled in April 2009. We did not determine

whether the soil seed bank of *P. floribunda* was transient or persistent, but judging from previous seed bank studies in rain forests of HAVO, the transient pattern is more likely (Drake 1998, Loh and Daehler 2008).

Seed germination of *C. giffardii* was highly variable, and much higher germination was observed on filter paper in the laboratory than was seen in seeds sowed on a mixed medium in the greenhouse, under conditions that might be considered a closer approximation of natural conditions in the forest. Roelofs (1979) also reported variable germination ranging from 0 to 90% in *Cyrtandra grandiflora* on O`ahu. The number of seeds per fruit counted for *C. grandiflora* in Roelofs's study ranged from 476 to 3,834. The low number of seeds per fruit in Roelofs's study is equivalent to the highest number counted for *C. giffardii* (and likely hybrids) in our study; most of the seed counts for *C. grandiflora* were more than 10 times those observed in *C. giffardii*.

Seeds of other *Cyrtandra* species lack dormancy, and the time for 50% germination reported for these other species was three weeks (Baskin *et al.* 2004). Plant propagators caution that *Cyrtandra* seeds lose viability quickly (Lilleeng-Rosenberger 2005). By contrast, seedlings observed in our greenhouse germination trials typically did not appear for nine months after sowing. Because seedlings were tiny and appeared among mosses that colonized the potting medium in the greenhouse, it is possible that seedlings were overlooked and actually appeared earlier. The low number of seedlings and the delayed germination we observed may indicate that the medium used was not optimum for *Cyrtandra* germination. We did not sample the soil seed bank for this species.

The seed germination rate for *S. alba* was relatively low, and two of the trials exhibited no germination. Other members of the genus *Sicyos* are known to have a combinational physical and physiological dormancy (Baskin *et al.* 2004). Physiological dormancy usually requires a time period of embryo maturation before the seed or fruit coat becomes permeable to water (Baskin and Baskin 1998). Our technique of clipping the thick inner fruit wall did not increase germination rates, unlike the results with the related *S. macrophyllus*, which responded to clipping the fruit and peeling out the seed with higher germination rates (Pratt *et al.* 2010). No soil seed bank was detected for *S. alba*. This lack may be natural, as large seeds with rough seed coats do not typically form persistent soil seed banks (Baskin and Baskin 1998). However, the appearance of natural seedlings many months after the disappearance of adult vines at one

monitoring site indicated that at least a transient soil seed bank was present (although undetected) at some of the *S. alba* plants in `Ōla`a.

Seedlings appeared to be a vulnerable life stage for both *P. floribunda* and *S. alba*, but we have no evidence for the cause of the loss of these seedlings. There was no obvious seasonal pattern to the timing of disappearance of the seedlings, and loss of seedlings was not associated with dry summer weather or cool winter temperatures. Seedlings of both species were primarily lost when they were small and less than three months old. The early loss of small seedlings of species with soft leaves suggests possible predation by alien slugs (Joe and Daehler 2008). We did not observe obvious signs of slug damage to seedlings, such as partial leaves or slug slime; seedlings disappeared totally rather than declining in vigor. Alien slugs, such as *Limax maximus*, the leopard slug, have been reported to have dense populations in `Ōla`a Forest (Dean 2006). Further studies using recently-developed, effective slug exclosures (Joe and Daehler 2008) around natural or planted seedlings may help determine the cause of seedling loss in `Ōla`a Forest.

Impacts of Rodents

Rodents, most likely rats (*Rattus* spp.) based on the size of tooth marks (Wilmshurst and Higham 2004), were confirmed as seed predators of *Sicyos alba* in `Ōla`a Forest. We never observed rodent damage to plants or fruits of either *C. giffardii* or *P. floribunda*, but we did not test predation with seed or fruit offerings. Other species of *Cyrtandra* on O`ahu were found to have low vulnerability to rat predation (A. Shiels, pers. comm.). Rodents were not listed as reasons for the decline of *C. giffardii* in that species' recovery plan (U.S. Fish and Wildlife Service 1996).

Rat damage was not observed to *S. alba* fruits still attached to the vines, but the pendent fruit borne on slender stems may have been inaccessible to rodents. The slender branches of canopy trees are generally considered less accessible to arboreal rats (Woodworth and Pratt 2009). On Maui, there is evidence that rats are attracted to the flesh surrounding seeds, making them more vulnerable to predation by rats when their fruit flesh is intact and when they are near the parent plant (Chimera 2004). Rats did not seem to be attracted to the flesh of *S. alba*, since fruits were not depredated in our offerings until after the fleshy fruit had deteriorated.

We did not carry out rat trapping as part of the current study, and therefore did not confirm the identity of the depredating rat. However, the rodent species composition of `Ōla`a Forest and nearby forests was dominated by black rats (*Rattus rattus*) in previous studies (Spurr *et al.* unpublished, Forbes and Stone unpublished, Tomich and Bridges 1981). Prevalence of black rats at middle- to high-elevation rain forests is typical in Hawai`i (Stone 1985, Tomich 1986). The house mouse, *Mus domesticus*, is rare in upland wet forests (Tomich 1986).

Rats are well known as seed predators in Hawai`i. Rat diet studies have identified fruit and seeds as important components of the food consumed in wet forests on Maui, where Sugihara (1997) identified species of *Rubus* (`ākala), *Pittosporum* (hō`awa), and *Coprosma* (pilo) in the stomach contents of rats he examined. Other forest and woodland species are also affected by rats. Cole *et al.* (2000) found that fruits and seeds of native woody species were consumed by rats in native shrubland of Haleakalā on Maui. In dry subalpine forests of Mauna Kea, black rats predated fruits and seeds of the native trees *Myoporum sandwicense* (naio) and *Sophora chrysophylla* (māmane), as well as alien grass seeds (Amarasekare 1994). Cabin *et al.* (2000) implicated rats as seed predators of *Diospyros sandwicensis* (lama) in dry forest of leeward Hawai`i.

Hybridization in *Cyrtandra* and Potential Management Strategies

The results of our morphology study of monitored *Cyrtandra* suggested that most of the plants in the study area population were actually hybrids, even though they had floral characteristics of the endangered *C. giffardii*. One hybrid that has *C. giffardii* as one of its parents has been recognized at lower elevations on Hawai`i Island, although this entity (formerly known as *C. ramosissima*) is different morphologically from the plants we observed in `Ōla`a. *Cyrtandra lysiosepala*, the most common *Cyrtandra* of `Ōla`a Forest, is known to hybridize with two other species in the Kūlani and `Ōla`a area (Wagner *et al.* 1999). Although we speculate that *C. lysiosepala* is one of the parents of the putative hybrids we studied, this species has not been confirmed to hybridize with *C. giffardii* elsewhere (Wagner *et al.* 1999). At least 67 *Cyrtandra* hybrids have been recognized in the Hawaiian Islands, and all that have had their chromosomes counted have $2n = 34$, the same number as all *Cyrtandra* species that have been studied (Carr 1998, Kiehn 2005). Chromosome anomalies have not been noted with *Cyrtandra* hybrids, and pollen stainability is high (Carr 1998).

It is not well understood how separate sympatric species are maintained in the presence of easily-formed hybrid populations (Wagner *et al.* 1999). However, the presence of several *Cyrtandra* species in `Ōla`a Forest and their formation of hybrids is a natural situation. If HAVO managers wish to increase the numbers of *C. giffardii* in the park, it may be possible to carry out vegetative propagation of individuals with strong characters of the endangered species and plant them at a site lacking other *Cyrtandra* species. Cuttings have been used as a propagation technique for other species in the genus (Lilleeng-Rosenberger 2005). The Kīlauea summit forests near Thurston Lava Tube (Nāhuku) provide possible sites for implementation of this strategy, as the area is part of a managed Special Ecological Area and the naturally occurring *C. platyphylla* (`ilīhia) is relatively uncommon and absent from many sites. The Kīlauea forests are within the original range of *C. giffardii* (Rock 1919). Some sites supporting *C. giffardii* outside the park are at lower elevations than `Ōla`a or Kīlauea summit, indicating that the lower-elevation forests of the East Rift Special Ecological Area may also be suitable planting sites for the species.

CONCLUSIONS

Limiting Factors of Three Rare Plant Species in `Ōla`a Forest

Cyrtandra giffardii. The `Ōla`a population (including hybrids) was relatively stable with a low adult mortality rate. Most plants with floral characteristics of *C. giffardii* were likely natural hybrids with the more common species *C. lysiosepala*. Successful fruit production was relatively high, but seed germination was low in the greenhouse. Low seed germination rates may indicate non-viable seeds and less than efficient pollinators or may be a reflection of inadequacy of artificial propagation techniques. Natural seedlings were not observed. Although pollinators were not observed in this study, the presence of apparent hybrids in the population means that some vector does affect pollination. Cross-pollination may be an uncommon event.

Phyllostegia floribunda. Mortality of the re-introduced population was relatively low, and growth in height and number of stems was observed during three years of monitoring. Reproduction of older plantings was successful with high rates of fruit formation from abundant flowers. Among insect floral visitors, the honey bee was the one most likely to pollinate flowers. However, the floral visitation rate of bees was very low. Seed germination rates were high, and a soil seed bank was detected. Natural seedling recruitment was observed, but

seedling mortality was high. Planted seedling mortality was also high. The seedling life stage appeared to be vulnerable to great loss in this species.

Sicyos alba. The rate of successful fruit formation from flowers was not confirmed but was suggested by the level of fruit production at monitored plants. Natural dormancy was likely a factor in observed low seed germination, which was not due to lack of seed viability. No soil seed bank was detected other than one predated seed, and alien rodents (most likely *Rattus* sp. based on tooth marks and the rarity of mice in montane wet forests) were implicated as seed predators in fruit offerings. Natural seedling recruitment was observed at multiple sites, but seedling mortality was high. Planted seedlings did not persist longer than 16 months.

Recommendations for Future Work

Cyrtandra giffardii. Improved techniques for seed germination and seedling production would increase the amount of propagation material available for restoration activities. Development of vegetative propagation techniques would allow the establishment of additional populations of "pure" *C. giffardii* at sites lacking other *Cyrtandra* species, thus reducing the likelihood of hybridization of the endangered species. Additional studies to determine if the species is self-compatible and if it is capable of self-fertilization might provide useful information. Identification of potential pollinators is important for understanding the reproductive system of the endangered plant and its propensity for hybridization, but results from the current study indicate that this might be a very time-consuming process.

Phyllostegia floribunda. Further investigation of the causes of natural seedling mortality is warranted. Experimental exclusion of alien slugs from natural or planted seedlings might help identify seedling predators and help managers develop strategies to promote seedling recruitment and population stability for this rare species.

Sicyos alba. Experimentation with techniques to increase seed germination rates is warranted to obtain plants for augmentation or introduction to new sites in `Ōla`a Forest. Evaluation of the role of alien slugs in causing seedling mortality might provide information important for the conservation and management of this species. Experimental reduction of rat populations in the vicinity of natural *S. alba* plants might establish the relative importance of

rodents in limiting seedling recruitment; such management might result in increased seedling and adult plant establishment.

Management Recommendations

- Effective control of alien rats during the periods of peak fruit production would likely benefit *S. alba*.
- The continued propagation and planting of *P. floribunda* and *S. alba* in additional sites at `Ōla`a will augment newly planted populations with young plants, increase the number of populations, and improve chances of long-term survival of the species in HAVO.
- *Cyrtandra giffardii* and *P. floribunda* could be considered for use in restoration projects at lower elevations in forests of Kīlauea's summit and East Rift, as both rare species are known historically from these areas.
- If alien slugs are identified as important seedling predators to *P. floribunda* and *S. alba*, management strategies to exclude them from natural seedling sites and planting sites may promote seedling recruitment and stability of populations.

ACKNOWLEDGEMENTS

This research was made possible by support from the Natural Resources Protection Program (NRPP) and the U.S. Geological Survey, Wildlife - Terrestrial & Endangered Resources Program.

We would like to acknowledge personnel of the U.S. Geological Survey, Pacific Island Ecosystems Research Center (PIERC), including federal administrative staff and volunteers, as well as personnel hired by the Research Corporation of the University of Hawai`i and administered through the Hawai`i Cooperative Studies Unit (HCSU), Pacific Aquaculture and Coastal Resources Center, University of Hawai`i at Hilo. We thank Faith Inman, formerly of HCSU, for her assistance during the first years of the project. We are grateful to interns who helped us at different times throughout the study: Sam Terpstra, Benjamin Gosney, Jennifer Johansen, Tyson Kemper, Ali Reiner, Melissa Holman, Nate Renie, Dan Bennett, Kolea Zimmerman, Grant Tolley, Alison Parker, Corinna Pinzari, Laura Stead, Laura Petteway, and Alex Cunningham. Without their help, we could not have accomplished the field monitoring, data collection, and data entry aspects of the project.

Plant propagation and restoration specialists of HAVO Natural Resources Management Division (NRM) and Pacific Cooperative Studies Unit, University of Hawai`i at Mānoa carried out several seed germination trials for this study and maintained resulting plants for use in experimental plantings and park restoration projects; we especially acknowledge Thomas Belfield, Joy Hosokawa, Sierra McDaniel, and Susan Dale. Thomas Belfield and Sierra McDaniel also shared with us their data on germination rates and planting success for our study species. Rhonda Loh, Howard Hoshida, and J. Timothy Tunison (retired) of HAVO NRM provided us with information on management activities within the `Ōla`a Forest management units. Aaron Shiels of the Botany Department, University of Hawai`i at Mānoa, shared with us his data on seed predation by rats.

We greatly appreciate the assistance of several specialists who identified insect specimens collected during pollination studies: Jon Giffin of The Nature Conservancy of Hawai`i; Karl Magnacca of the University of Hawai`i at Hilo; Curtis Ewing of the University of California at Berkeley; and Dan Polhemus of the Hawai`i Department of Land and Natural Resources. Staff of the Bernice P. Bishop Museum in Honolulu facilitated our use of the entomology collections. Robert Peck of the Hawai`i Cooperative Studies Unit advised us on entomological aspects of the pollination work. We also thank Steven Hess of the U.S. Geological Survey, PIERC, and Marie Bruegmann of the U.S. Fish and Wildlife Service, Ecological Services, Honolulu, for constructive criticism of this manuscript.

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APPENDIX I

Descriptions of the Flowers of Three Study Species in `Ōla`a Forest

Cyrtandra giffardii. Flowers are borne in axillary inflorescences of three to five flowers. The inflorescence, a cyme, is covered with short brown hairs and has slender peduncles 25-35 mm long and unequal pedicels 15-30 mm. Bracts are linear and 6-7 mm long. Individual flowers have a small calyx 3-9 mm long, cleft to near the base, with narrow lobes. The tubular flower is white with a corolla 12 mm long and short corolla lobes 2-3 mm long. A nectary disk surrounds the superior ovary. The style is 2 mm long, glabrous, and topped with a two-lobed stigma. There are five stamens inserted on the corolla tube; the upper two are typically fertile with the anthers positioned in the throat of the corolla tube, and the others are staminodes with abortive anthers (Wagner *et al.* 1999). The flowers are open in both the day and night and appear to have no scent in the day.

Phyllostegia floribunda. Flowers are borne in pairs on racemose inflorescences with short axillary stalks covered with hairs. Individual flowers have pedicels 8-10 mm long with a bract, obovate in shape and 3-5 mm long. The calyx of the flower is obconical, 2-5 mm long, with 10 veins or nerves; it is covered with glandular hairs and gland dots. Calyx teeth are linear-lanceolate, 1.2-2.5 mm long, with a long tapered tip. The corolla is maroon or red with long, soft hairs. The corolla tube is straight, 8-10 mm long; the small upper lip is 2.5 mm; and the lower lip is 4-5 mm long. There are four stamens with one pair longer than the other but still within the corolla. Stamen filaments are finely hairy and bear anthers with two cells. The pistil has a two-lobed style included within the corolla and a terminal stigma (Wagner *et al.* 1999). Flowers are slightly fragrant.

Sicyos alba. Flowers are unisexual, and male and female flowers are borne on separate inflorescences at the same leaf node. Male inflorescences are panicles up to 7 cm long with peduncles of 2.5-4 cm; inflorescences contain many small flowers. Pedicels of individual flowers are 2-4 mm long and are smooth and hairless. The male corolla (perianth) is white, gland-dotted, to 2.5 mm in diameter, and five-lobed. Within the male flower are three to five stamens coiled together, bearing anthers twisted and exserted from the corolla. Female (pistillate) flowers are borne in round heads with a peduncle 1-3.5 cm long. There are usually 2-8 flowers per head. Female flowers have a green, tubular-shaped base 5 mm long and four white corolla lobes 1 cm long. No staminodes are present, and the central style is 1.3 mm long with its two-lobed stigma exserted from the flower (Wagner *et al.* 1999, St. John 1978b).

APPENDIX II

Data from Greenhouse Germination Trials and Seed Predation Experiment in `Ōla`a Forest, HAVO

Table 1. Greenhouse seed germination trials for *Cyrtandra giffardii*.

Plant/ Replicate#	#Seeds	Date Sown	Date 1 st Germination	# Seeds Germinated	% Germinated	Date Terminated
Petri/1	50	5/30/06	6/15/06	30	60.0	7/18/06
Petri/2	50	5/30/06	6/15/06	36	72.0	7/18/06
38/1	50	10/10/06	n/a ¹	0	0.0	6/18/07
38/2	50	10/10/06	Ca. 3/8/07	6	12.0	6/18/07
38/3	50	10/10/06	n/a ¹	0	0.0	6/18/07
32/1	50	10/10/06	n/a ¹	0	0.0	6/18/07
32/2	50	10/10/06	n/a ¹	0	0.0	6/18/07
32/3	50	10/10/06	n/a ¹	0	0.0	6/18/07
9/1	*	11/20/07	n/a ¹	0	0.0	6/26/09
20/1	223	5/23/08	n/a ¹	0	0.0	6/26/09
23/1	48	2/21/08	n/a ¹	0	0.0	6/26/09
23/2	134	2/21/08	11/14/08	24	17.9	6/26/09
23/3	*	2/3/08	12/5/08	25	10.7*	6/26/09
28/1	*	6/1/07	No data ²	33	14.2*	6/26/09
28/2	*	2/3/08	No data ²	1	0.4*	6/26/09
28/3	*	2/3/08	12/29/08	45	19.3*	6/26/09
28/4	*	2/3/08	n/a ¹	0	0.0	6/26/09
28/5	*	2/3/08	n/a ¹	0	0.0	6/26/09
28/6	*	2/3/08	n/a ¹	0	0.0	6/26/09
28/7	440	2/20/08	11/14/08	4	1.0	6/26/09
28/8	73	2/20/08	n/a ¹	0	0.0	6/26/09
28/9	99	5/23/08	n/a ¹	0	0.0	6/26/09
31/1	*	10/23/07	No data ²	3	1.3*	6/26/09
31/2	343	2/21/08	12/19/08	2	0.6	6/26/09
33/1	502	5/22/08	n/a ¹	0	0.0	6/26/09
33/2	302	5/22/08	n/a ¹	0	0.0	6/26/09
40/1	*	6/1/07	No data ²	23	9.9*	6/26/09
40/2	162	5/22/08	n/a ¹	0	0.0	6/26/09
40/3	128	5/22/08	n/a ¹	0	0.0	6/26/09
50/1	185	5/22/08	n/a ¹	0	0.0	6/26/09
50/2	394	5/22/08	n/a ¹	0	0.0	6/26/09
60/1	*	2/3/08	n/a ¹	0	0.0	6/26/09
65/1	*	11/20/07	n/a ¹	0	0.0	6/26/09

* Seeds of fruit not counted, mean number of seeds/fruit (233) used to calculate germination rate.

¹ Not applicable, no germination observed.

² No data, date of first germination not recorded.

Table 2. Greenhouse seed germination trials for *Phyllostegia floribunda*.

Trial/Rep.#	#Seeds	Date Sown	Date 1 st Germination	# Seeds Germinated	% Germinated	Date Terminated
3/1	25	7/6/06	8/17/06	22	88.0	1/23/07
3/2	25	7/6/06	8/17/06	25	100.0	1/23/07
3/3	25	7/6/06	8/17/06	25	100.0	1/23/07
3/4	50	7/6/06	8/17/06	50	100.0	1/23/07
3/5	25	7/6/06	8/17/06	14	56.0	1/23/07
3/6	25	7/6/06	8/17/06	12	48.0	1/23/07
3/7	50	7/6/06	8/17/06	50	100.0	1/23/07
6/1	50	6/12/08	No data ¹	40	80.0	3/13/09
6/2	50	6/12/08	No data ¹	48	96.0	3/13/09
6/3	50	6/12/08	No data ¹	50	100.0	3/13/09
10/1	56	7/29/08	9/2/08	48	85.7	4/29/09
10/2	56	7/29/08	9/2/08	47	83.9	4/29/09
10/3	56	7/29/08	9/2/08	50	89.3	4/29/09
10/4	56	7/29/08	9/2/08	53	93.0	4/29/09

¹ No data, date of first germination not recorded.

Table 3. Results of *Sicyos alba* seed offerings in open and closed rat bait stations after three months in `Ōla`a Forest, HAVO.

Replicate	Treatment	# Seeds	Date	# Seeds Predated	# Seeds Missing
1	open	10	4/17/2009	1	7
1	closed	10	4/17/2009	0	0
2	open	10	4/17/2009	7	3
2	closed	10	4/17/2009	0	0
3	open	10	4/17/2009	6	4
3	closed	10	4/17/2009	0	0

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

Trial/Rep.#	#Seeds	Date Sown	Date 1st Germination	# Seeds Germinated	% Germinated	Date Terminated
10/1	25*	1/18/07	2/25/07	2	8.0	8/7/07
10/2	25*	1/18/07	2/25/07	1	4.0	8/7/07
10/3	25*	1/18/07	n/a ¹	0	0.0	8/7/07
11/1	18	7/24/07	No data ²	1	5.5	7/18/08
11/2	18	7/24/07	No data ²	3	16.7	7/18/08
11/3	18	7/24/07	No data ²	2	11.1	7/18/08
12/1	6	10/3/07	10/31/07	1	16.6	7/18/08
13/1	33	2/21/08	n/a ¹	0	0.0	2/6/09
13/2	33	2/21/08	n/a ¹	0	0.0	2/6/09
13/3	33	2/21/08	n/a ¹	0	0.0	2/6/09
14/1	22*	1/20/09	n/a ¹	0	0.0	12/31/09
14/2	22*	1/20/09	n/a ¹	0	0.0	12/31/09
14/3	22*	1/20/09	n/a ¹	0	0.0	12/31/09

*Inner fruit wall clipped to seed.

¹ Not applicable, no germination observed.

² No data, date of first germination not recorded.