Holocarpha macradenia Greene (Santa Cruz tarplant) Population Enhancement Project, 2005-2006 Twin Lakes State Beach, Santa Cruz, CA

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The objective of this project was to increase the population size and soil seed bank density of *Holocarpha* macradenia at Twin Lakes State Beach, Santa Cruz County, California (Figure 1). Two cohorts (2005 and 2006) of H. macradenia were cultivated, outplanted and monitored. Before this project, the population was small (<20 individuals), and adjacent to a dirt road. In addition, the density of the seed bank was magnitudes less than other populations, and small enough to put this population at risk.

Methods: Approximately 1,350 heads (<5% total heads in population) of *Holocarpha macradenia* were collected in September and October from each of the 2002-2005 outplant areas (Figures 1 and 2). Collection and storage tracked cohorts, individuals and heads. In October 2005 and 2006, achenes from these collections were germinated in Petri dishes. Seedlings were transferred to 2.75 by 2.75 inch or 3.25 x 3.25 inch pots, and grown for 1-2 weeks, in a growth chamber, and then transferred to a greenhouse for another 4-6 weeks before outplanting. Achenes were chosen to maximize as much genetic diversity as possible, using cohorts, individuals and heads on individuals as proxies for genetic diversity, or at least parentage and therefore s-alleles. These factors (cohort, individuals, and heads) were tracked during propagation so that outplanting pattern would represent genetic heterogeneity.

In February 2005, 736 seedlings were outplanted in four areas (2005 a-d on Figure 2). In January and February 2006, 831 seedlings were outplanted in five areas (2006 a-e in Figure 2). To reduce snail predation, Sluggo (W. Neudorff GmbH KG, Germany) was applied at the time of planting and then weekly, 3-4 times after the planting date, up until early April or May. Earlier experiments showed a significant increase in seedling survivorship when Sluggo was used at the time of outplanting. Non-native vegetation between the outplanted seedlings was hand weeded two to four times after outplanting. In both years, rainfall was consistent and adequate to maintain soil moisture. Supplemental water was not added.

In January 2006, 120 disc achenes were outplanted in six plots throughout the site. The plots were hand weeded prior to outplanting achenes. Achenes were placed within the first cm of soil with tweezers and the achenes were watered.

Survivorship of the outplanted individuals was monitored 9 times per cohort by counting each individual. The mean number of heads (inflorescences) per plant in the outplanted populations were estimated in mid-August from approximately every other plant in each plot. Heads were harvested and viable seeds/head was estimated. Seeds were used for outplanting.

The number and distribution of naturally recruiting plants in each of the outplant areas from previous years was determined by flagging each plant or cluster of plants, and counting individuals (e.g., Figure 3). The mean number of heads per plant in the natural recruiting population was estimated by counting heads per plant on each plant. The subset of heads were harvested to estimated seeds/head and then use for outplanting.

Results. Numbers of outplanted or naturally recruiting individuals in each outplant area by September 24, 2005 and the mean numbers of heads per plant are reported in Table 1a. These results for 2006 are reported in Table 1b.

Survivorship of outplanted seedlings through to seed maturation in 2005 was 93%, and 98% in 2006 (Figure 4). Most mortality occurred within a month of outplanting. This mortality probably represents those individuals exhibiting poor vigor at the time of outplanting. After that, minor (<1%) additional mortality occurred prior to maturation of achenes and natural senescence of plants. Numbers of individuals in each outplant area by mid to late-September, and the mean numbers of heads per plant are reported in Table 1 (2005) and Table 2 (2006). Figures 5 and 6 show plants in the 2005 outplant areas between mid-June and September, 2005. Recruitment from outplanted seeds was

Plants in the outplanted area were large (Figure 5) and the number of heads per plant ranged from one to 115. In both years, the mean numbers of heads on the outplanted plants were significantly higher than on the natural recruits. The number of heads per plant was not significantly different between outplant areas, in either year. The number of ray achenes per head in the outplanted plants from seedlings was 7.6 and the number of disc achenes was 6.4 in 2005; and 5.9 for ray achenes and 5.5 for disc achenes in 2006 (n=30 heads/year); and 4.1 rays and 2.8 discs on the plants outplanted as seed (n=15 heads). The number of ray achenes per head was 4.3 and the number of disc achenes was 3.0, in 2005 on the natural recruits; and in 2006, the number of ray achenes per head was 4.6 and the number of disc achenes was 3.5 (n=30 heads/year).

In 2005, over 600 individuals recruited in the 2002-2004 outplant areas (Table 1, 2002/2004, 2003a-b, and 2004 a-c in Figure 2, Figure 3), and three individuals recruited along the dirt road in the area of the original population ("original population" in Figure 2). In 2006, no recruitment occurred in the original population but 698 individuals recruited in former outplant areas. In both years. phenology of the outplanted population was slightly ahead of the natural recruiting individuals due in part to greater size of outplants at the time of outplanting in comparison to the natural recruits.

Table 1a. Number of individuals (September 2005), mean and total heads per plant (August 2005).

	Outplanted or natural recruitment in 2005?*	# individuals	Mean heads/plant	Total heads
2005a	Outplanted as seedling	158	49.9	7,879
2005b	Outplanted as seedling	181	42.6	7,720
2005c	Outplanted as seedling	218	36.8	8,025
2005d	Outplanted as seedling	130	15.2	1,979
Original	Natural	3	6.3	19
2004a-c	Natural from outplant	474	4.4	2,034
2002/2004	Natural from outplant	23	4.5	104
2003a	Natural from outplant	56	4.6	257
2003b	Natural from outplant	80	4.5	357
Total outplanted	**Outplant**	**687**	**37.3**	**25,603**
Total natural	**Natural**	**636**	**4.4**	**2,771**
TOTAL	**Outplant and natural**	**1,323**	**21.4**	**28,374**

Table 1b. Number of individuals (September 2006), mean and total heads per plant (August 2006).

	Outplanted or natural recruitment?*	# individuals	Mean heads/plant	Total heads
2006a (at 2005a)**	Outplanted as seedling	148	38.1	5,638
2006b (at 2005b)	Outplanted as seedling	162	36.6	5,929
2006c (at 2005c)	Outplanted as seedling	225	43.8	9,855
2006d	Outplanted as seedling	110	29.5	3,245
2006e	Outplanted as seedling	166	31.1	5,162
2006 a-e	Outplanted as seed	42	3.9	164
Original	Natural	0	n/a	n/a
2005a	Natural from outplant	91	16.4	1,492
2005b	Natural from outplant	45	13.0	585
2005c	Natural from outplant	89	11.9	1,059
2004a-c	Natural from outplant	331	3.8	1,258
2002/2004	Natural from outplant	17	4.4	75
2003a	Natural from outplant	76	3.7	281
2003b	Natural from outplant	49	3.1	152
Total outplanted	**Outplant from seedling**	**811**	**36.7**	**29,829**
Total outplanted	**Outplant as seed**	**42**	**3.9**	**164**
Total natural	**Natural from outplant**	**698**	**7.0**	**4,902**
TOTAL	**Outplant and natural**	**1,551**	**22.5**	**34,895**

*Outplant=seedlings outplanted in 2005; Natural=existing/original population; Natural from outplant = areas outplanted in previous years with natural recruitment in 2005.
** 2006 outplants at 2005 outplant site

Discussion and Recommendation: In both years, survivorship and reproductive output of ouplanted individuals, and seed input into the soil seed bank was high; on the order of (approximately 358,400 achenes in 2005, and 359,352 in 2006). This indicates a return of approximately 485 achenes in 2005 and 408 in 2006, for each one used in the seedling outplanting. And, 9.4 achenes for each one used in the seed outplanting. It also indicates that there are not genetic limitations to reproductive output. Previous surveys of the soil seed bank and for recruitment in these areas indicated that neither occurred prior to outplanting. Natural recruitment (636-698 individuals) in the outplant areas is 24-46 times the previous recruitment numbers, and suggests that at least a transient soil seed bank has been established. However, accumulation in the persistent soil seed bank is probably magnitudes lower than seed input (Bainbridge, pers. obs,

other populations). Soil samples have been collected from the outplant areas to assess soil seed bank density.

The mean number of heads and achenes per head on the outplanted plants were significantly higher than on the natural recruits probably because of the size of the plants at time of outplanting was much larger (to 5 times) than the size of the natural recruits at the same time. Earlier trials varying outplanting sizes and dates indicated the importance of initial size, including root mass, in reproductive output. Reduced competition in the outplanting areas, due to weeding of non-native plants, was probably also a factor. Although natural recruits in outplant areas 2005a-c also experienced reduced competition through weeding.

In early November 2006, an accidental fire burned the entire population. At the time, much of the seed has dispersed from the plants and was most likely at the soil surface. Observations in winter and spring of 2007 indicate recruitment will not be as extensive as in 2005 or 2006. In addition, biomass in general, and specifically several non-native forbs (*Trifolium angustifolium*, *Vicia villosa*, *Hypocheris* spp., etc.) and *Briza major* appears to be higher than in previous years. This is possibly in response to fire. Recruitment of *Holocarpha macradenia*, and maintenance of the augmentation of this population from this effort, will require control of this biomass.

Figure 1. Location of *Holocarpha macradenia* outplanting project. a) Location of Twin Lakes State Beach in Santa Cruz, CA (map from Yahoo), b) dirt road in park, outplanting on both sides.

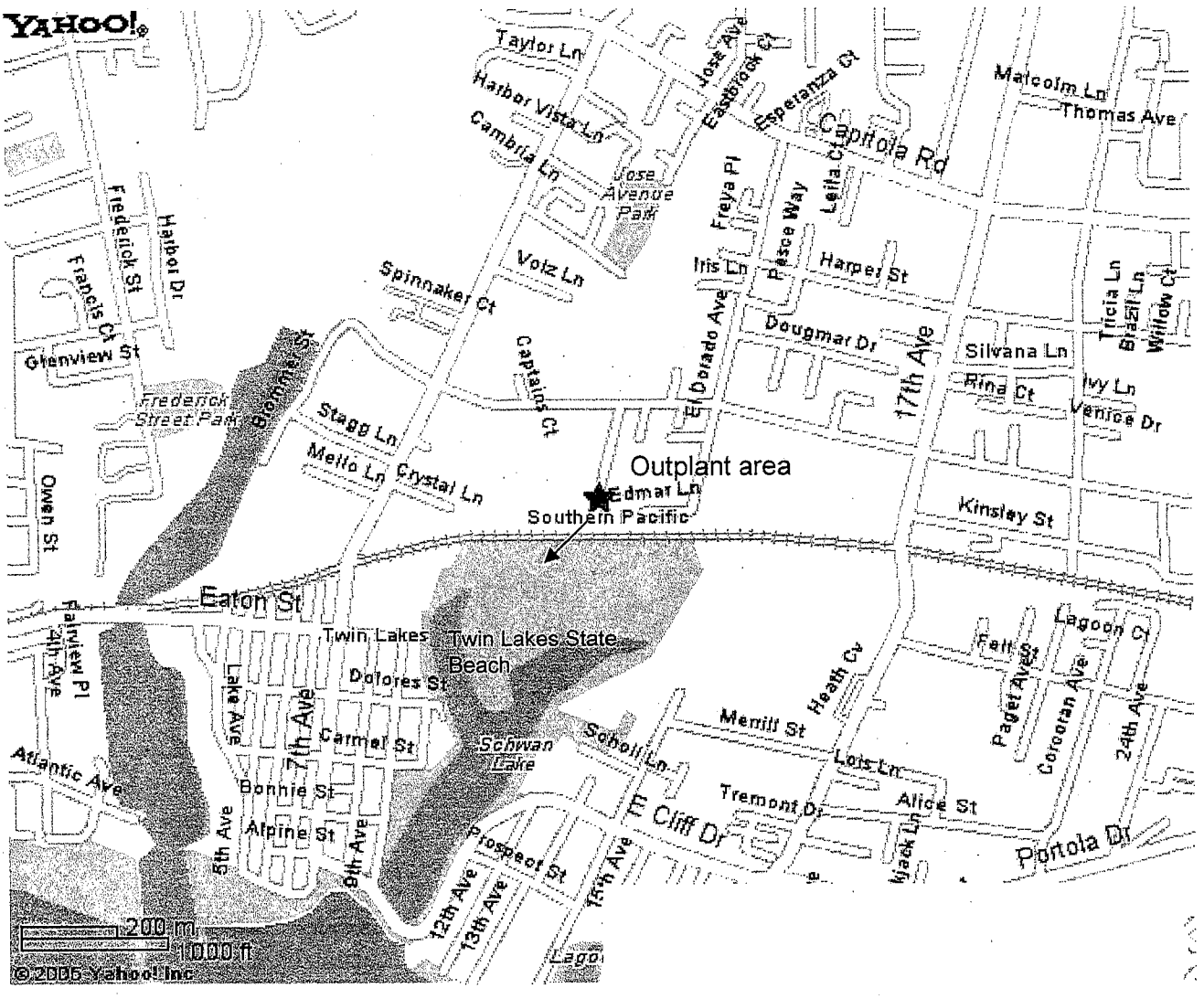

Figure 2. Locations of 2002-2006 *Holocarpha macradenia* outplanting areas and the original population, Twin Lakes State Beach, Santa Cruz County, California. Basemap from California Department of Parks and Recreation.

Figure 3. Distribution of naturally recruiting *Holocarpha macradenia* plants, in portion of a) 2003 and b) 2004 outplant area (flags represent clusters of plants), September 2005, Twin Lakes State Beach, Santa Cruz County, CA.

a)

b)

Figure 4. Survival of outplanted *Holocarpha macradenia* seedlings, Twin Lakes State Beach, February-October, 2005 and 2006.

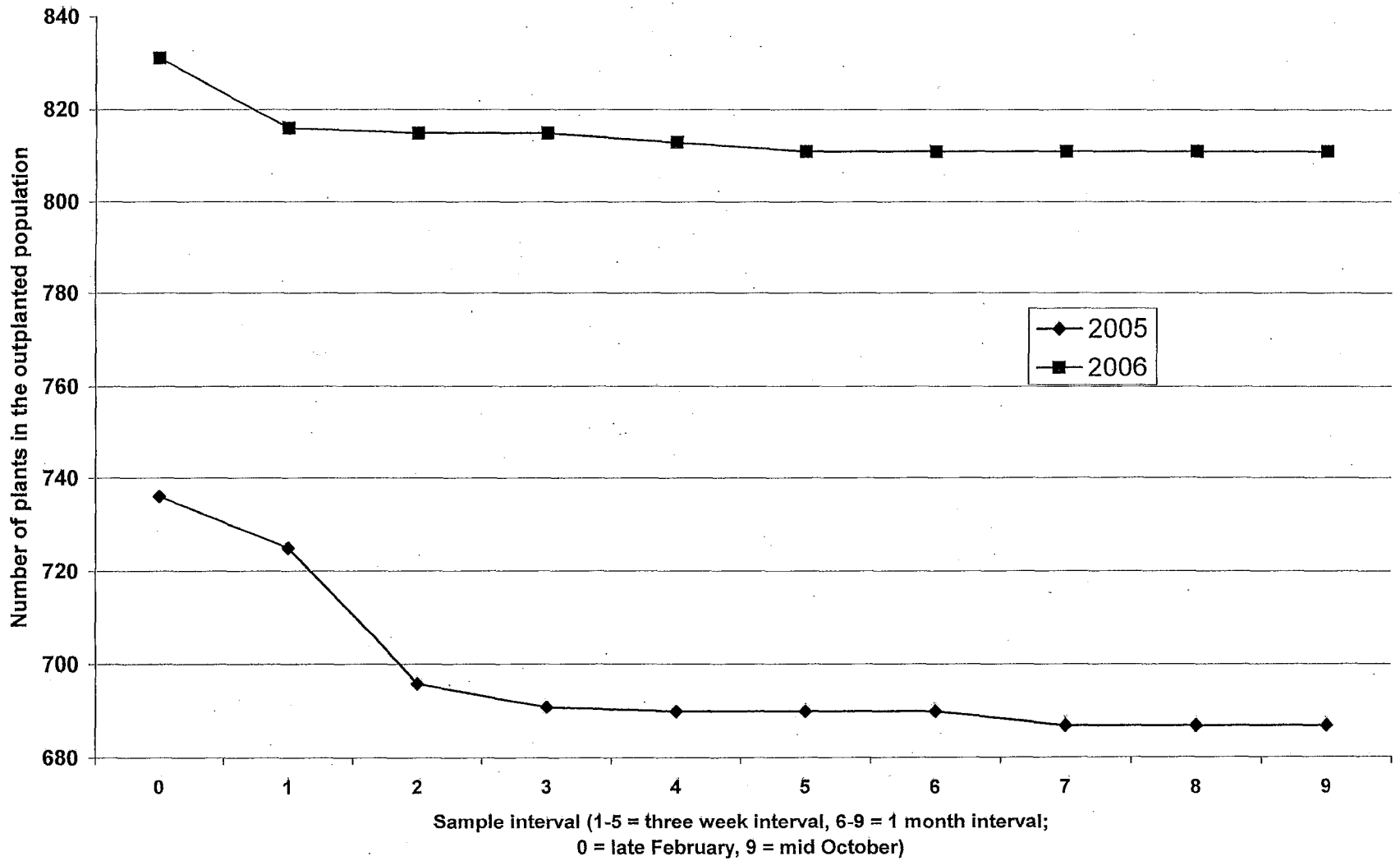

Figure 5. Outplanted *Holocarpha macradenia* plants at Twin Lakes State Beach, Santa Cruz County, CA, a - b) mid-June, c) August, and d) September 2005.

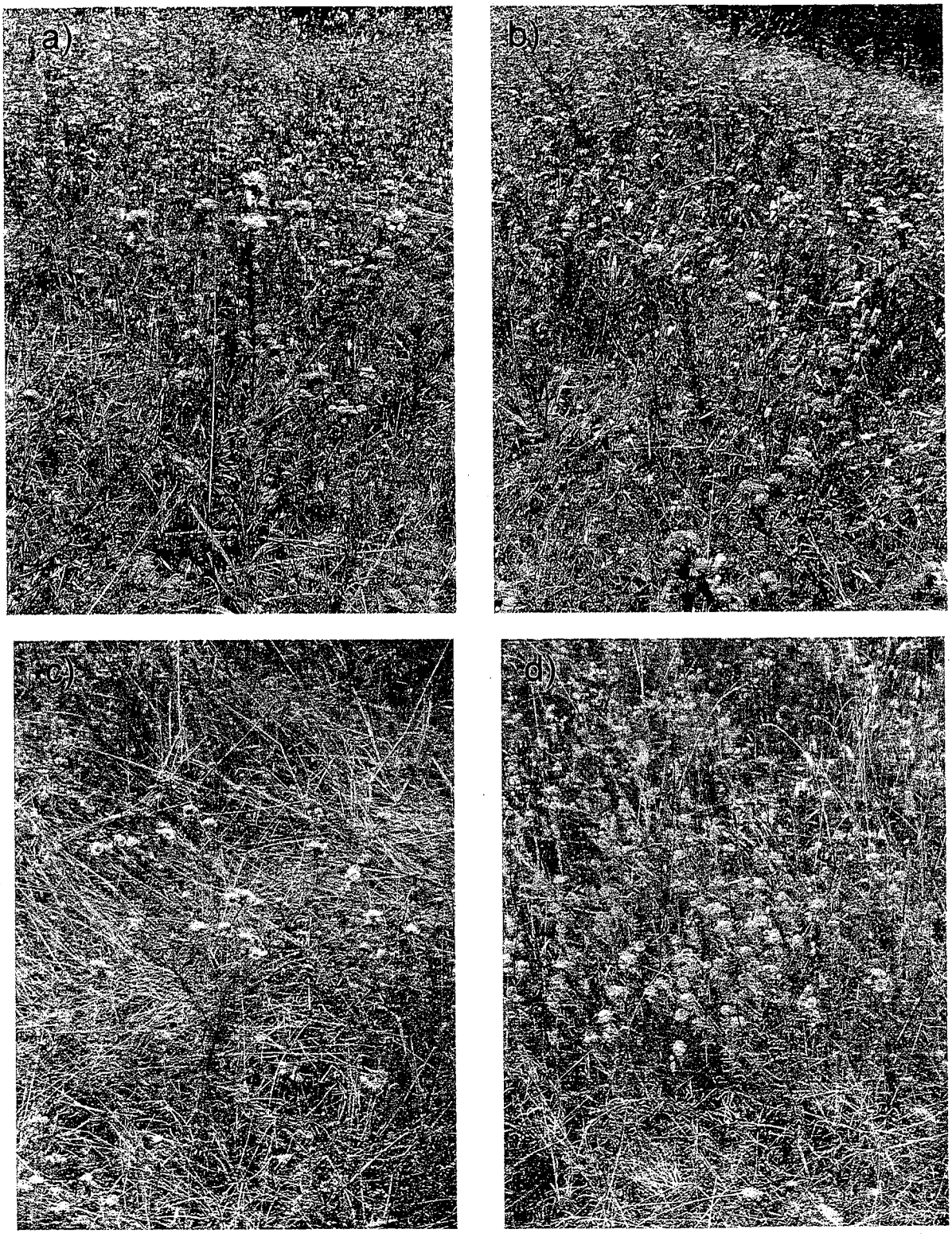

Figure 6. *Holocarpha macradenia* outplant areas a) 2005c and b) 2005a, August 2005, at Twin Lakes State Beach, Santa Cruz County, CA.

a)