Nutlet Production and Germination of *Amsinckia grandiflora*

I. Measurements From Cultivated Populations

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Abstract

The recovery of Amsinckia grandiflora will require a knowledge of fecundity, germination, and seedling growth in order to monitor the existing population and anticipate problems that could arise during the establishment of the proposed satellite populations. In order to extend the currrent monitoring and recovery program for A. grandiflora, this investigation sought to I) develop effective propagation and culture techniques to ensure that the maximum number of seedlings can be obtained from the available good seed, 2) develop predictive relationships between plant size (e.g plant height or infloresence length) and nutlet production, 3) establish a nutlet-producing population in the Mills Botanical Garden, and 4) ascertain the quality of the nutlets produced in the garden.

The methods used to cultivate Amsinckia grandiflora at Mills College were effective in amplifying the original population from 75 to 732 nutlets. The cultivated nutlets were generally small in size, but at least 25% exhibited the ability to germinate within two months of production. From a standpoint of efficiency, however, only 12% of the available ovules were converted into seeds. This could have been due to pollinator limitations linked to the number of effective pollinators in the vicinity of the Mills Botanical Garden. The Mills culture method is recommended when nutlets are in short supply and the population of effective pollinators can be enhanced. The methods used to cultivate Amsinckia grandiflora at UC Berkeley Botanical Garden were also effective with respect to nutlet production. Those nutlets were of a larger size than Mills nutlets, but they also possessed an additional dormancy mechanism that prevented an assessment of germination ability. It appears that the although the high density culture method reduced plant size and reproductive potential, reproductive efficiency was enhanced relative to the low density Mills method. The UC Berkeley culture method is recommended when nutlets are not in short supply (although data on establishment efficiency are lacking) and when pollinator populations may be

limited. From a standpoint of increasing the number of nutlets available for establishing new populations, the cultivation of Amsinckia grandiflora as carried out at UC Davis is probably the best way to amplify the existing supply of nutlets. This method is recommended when nutlets are not in short supply (although data on establishment efficiency are lacking).

Germination percentage of large nutlets (6 months post-production) was high and probably does not present a significant barrier to the establishment and growth of populations. A number of other phenomena observed in this study could, however, inhibit population growth. These included 1) "caging" and subsequent inhibition of seedling growth by attached pericarps, 2) a very eccentric pin/thrum ratio of plants germination from Davis nutlets, 3) high mortality in the stage between flowering and nutlet production, 4) inefficient conversion of ovules into seeds, and 5) the possible production of different kinds of nutlets with different dormancy mechanisms, resource reserves and competitive abilities. Whether or not these phenomena are important in situ will have to be tested during detailed demographic studies on new satellite populations.

There was a strong linear relationship between nutlet output and shoot length, shoot weight and total inflorescence length, meaning that larger plants, however assessed, were more fecund than smaller plants. The least amount of variability was found in the total inflorescence length-nutlet output relationship. The strength of these relationships indicates that useful estimates of in situ nutlet production can be obtained using non-destructive measurements of plant height or total inflorescence length. It is recommended, however, that additional data be obtained from field plants at Lawrence Livermore Laboratory's Site 300 or at the reintroduction site in order to increase the accuracy of the equations and their estimates.

Since nutlets can be effectively obtained by cultivation, it is recommended that a large quantity be obtained from a variety of sources (UC Berkeley, Mills and UC Davis) and used to establish a new population of Amsinckia grandiflora within its historic range. The results of this study indicate that during the establishment of the new population, it will be important to favor vegetative growth and the production of large plants while increasing reproductive efficiency. This could be accomplished by

sowing nutlets directly into deep soil, controlling competition from annual grasses (controlled burning, grass herbicide, hand weeding) and selecting a site with good potential for supporting populations of effective pollinators.

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Introduction

The population size of a plant species within a given area of habitat can be limited by the production of viable seeds, particularly when the species does not reproduce asexually. Amsinckia grandiflora, like all annual plants (Harper 1977), does not propagate asexually. Populations of this taxon, therefore, depend entirely on seed (= nutlet) production.

Some annuals have the potential of producing large amounts of seed in an average year. This could be due to the fact that 1) many ovules are formed within each flower (e.g. annual species of Eschscholtzia, Mimulus, Camissonia), 2) many flowers are produced per plant (Brassica, Lupinus), and/or 3) their pollination or sexual systems allows for an efficient conversion of ovaries into seeds. Such is not the case with Amsinckia grandiflora, which consistently produces only 4 ovules per flower (Ornduff 1976), has relatively few flowers per plant (Taylor 1987), and posseses an ancestral self-incompatibility mechanism (pin and thrum flower morphs) that results in less than 30% conversion of ovules to seeds in situ (Ornduff 1976) and 40% after hand pollination in the greenhouse (Weller and Ornduff 1977). In addition, fecundity in situ may be limited by resource competition with annual grasses that have come to dominate the habitat of Amsinckia grandiflora. Resource limitations (e.g. lack of water or mineral nutrients) inhibit vegetative growth, and therefore, the number of flower-bearing branches and ovule-containing flowers. Further reductions in nutlet production may occur because of pre-dispersal predation by birds (R. Kelley, UCD, personal communication).

The recovery of Amsinckia grandiflora will require a knowledge of fecundity, germination, and seedling growth in order to monitor the existing population and anticipate problems that could arise during the establishment of the proposed satellite populations. In order to extend the currrent monitoring and recovery program for A. grandiflora, this investigation sought to I) develop effective propagation and culture techniques to ensure that the maximum number of seedlings can be obtained from the available good seed, 2) develop predictive relationships between plant size (e.g plant

height or infloresence length) and nutlet production, 3) establish a nutlet-producing population in the Mills Botanical Garden, and 4) ascertain the quality of the nutlets produced in the garden.

Methods and Materials

Approximately 150 nutlets of Amsinckia grandiflora were obtained from Ronald Kelley (graduate student, Department of Environmental Toxicology, UC Davis) during the late summer of 1987. These were produced by plants grown in Davis during the spring of 1987. The original source of nutlets for the Davis population can be traced to Robert Ornduff's collections at Lawrence Livermore Laboratory's Site 300, San Joaquin County, in the early 1960's. All of the nutlets used in these studies were of good form and color, with an average weight of 3.82 mg. They were stored in a glass vial at room temperature until germination and propagation studies were initiated.

Germination. On 15 January 1988, 25 nutlets were placed in each of three sterile plastic petri dishes (5.5 cm diameter) on sterile filter paper disks and moistened with sterile distilled water. These were kept in a completely dark culture room throughout the trials. Air temperatures in the culture room fluctuated between 19 C and 10 C over a 24 hour period. Dishes were checked every day for 12 days, noting germination (protrusion of the radicle through the pericarp) and removing germinules with a soft paintbrush. These were placed in another petri dish with paper and water for observing growth and in preparation for transfer to pots.

Growth and development of seedlings. Once transfered from the germination dishes to the growth dishes, the germinules were observed and measured in order to document the formation of root hairs, the rate of radicle growth and the timing of cotyledon emergence from the pericarp. During this time they were maintained under the same conditions as the ungerminated nutlets (total dark, 19C/10C air

temperatures). After the emergence of any portion of the cotyledons, seedlings were given 12 hrs of light from a fluorescent light bank (maximum intensity at plant height = 250 umol m-2 s-1 PAR, about 1/8 of full sun). When the seedlings were 4-5 days old they were carefully transplanted to pots (see below) and kept beneath the light bank for additional observations of growth and development.

Transplantation to pots. 4-5 days after germination the seedlings (with root hairs and greening cotyledons) were transferred to peat pots (7.7 cm diameter, two seedlings per pot) containing compacted, sterile potting mix that had been water-saturated. A dissection needle was used to open a small hole, 2 cm deep into which the seedling could be inserted. Care was taken to prevent the collapse of root hairs by suspending a drop of water from the hypocotyl to the root tip in which the apical root hairs would be supported. When the seedling was positioned within the hole a stream of water was used to move the soil around the root and insure good contact. Seedlings were kept beneath the light bank for 14 hours/day, with air temperatures fluctuating between 18 C/12 C on a 24 hour period. Ten days after germination the seedlings were moved outside and exposed to natural fluctuations in light intensity and temperature. They were initially given distilled water every three days and 1/4 strength Hoagland nutrient solution every week. Approximately 55 days after germination the peat pots were placed in large plastic pots (15 cm diameter) and surrounded with additional potting mix. At this time the plants were large and leafy and required watering every other day. At the beginning of floral anthesis, 32 plants (designated as "M") were moved to the Mills College Botanical Garden and 18 (designated as "M-UC") to the UC Berkeley Botanical Garden in order to balance the pin/thrum ratio (which was greatly skewed towards pins, see below). M-UC plants were kept near the plants cultivated using UC's methods (these designated as "UC") and that had a more typical pin/thrum ratio. M-UC and UC plants, however, experienced a cooler, more humid microclimate than did M plants as a result of being raised in Berkeley rather than Oakland.

Nutlet (seed) production. Plants were harvested after growth had naturally ceased. Drying shoots were clipped at soil level, placed in open polyethylene bags and kept at

room temperature for four weeks to ensure adequate dehydration. Measurements of stem and inflorescence length (see Figure 7), weights of the shoot and reproductive parts (flowers + nutlets) and counts of the number of branches, inflorescences and flowers were made on this air-dried material. Inflorescences were removed from the vegetative portions of the plant by clipping immediately below the first flower. Each flower was examined for the presence of filled (good quality) nutlets which were then counted, removed, and placed in a pre-weighed envelope assigned to that individual plant. Nutlets from a single individual were weighed together and the average weight/nutlet was computed by dividing by the total number of nutlets. The number of ovules was estimated by multiplying flower number by 4 since each flower produces 4 single-ovuled nutlets (Ornduff 1976).

Linear and non-linear regressions of plant size vs. nutlet production were made using several measures of the former, including total plant length, total inflorescence length (the sum of inflorescence lengths from a single plant) and dry shoot weight. The relationship with the highest regression coefficient was used to express the effect of plant size on reproductive output in Amsinckia grandiflora. M and M-UC plants were analyzed separately in order to determine if differences in microclimate affected fecundity. It was found that there was no effect, so data from M and M-UC plants were pooled and referred to as "Mills" plants.

Assessment of nutlet quality. After harvest, seeds from each individual were sorted into two size categories by visual assessment (large and small) and weighed separately. A total of 20 seeds from each category (2 lots of 10) were randomly selected from each of the cultivated populations (UC 1988, Mills 1988) for germination trials as described above.

Comparison of plants grown with different cultural methods. A large number of Amsinckia grandiflora plants (designated as "UC") were grown at UC under the supervision of Dr. Jim Affolter using cultivation methods that differed from those used at Mills. Approximately 400 plants were grown at high density (132 plants/dm^2 vs. 11.3 plants/dm^2 Mills) in a single wooden planter measuring 55 cm X 55 cm X 30 cm

(depth). The nutlets were sown directly into the planter which contained UC mix covered by a thin layer of small gravel. They received water every third day. On April 13, 1988, after the plants had begun to senesce and dry naturally, 10 plants were harvested, individually bagged and brought to Mills for the analysis of nutlet production and quality as described above. Mills and UC plants were analyzed separately in order to determine if differences in culture technique affected vegetative growth and fecundity. It was found that many of the size - fecundity relationships were similar in the two artificial populations, so data from Mills and UC plants were sometimes pooled to increase sample size.

Results and Discussion

Germination. Nutlets of Amsinckia grandiflora readily germinated under laboratory conditions, with an average of 80.0% ± 3.3% germination after 10 days (Figure 1). Fungal contamination was low in the petri dishes and there was no obvious reason why the other nutlets did not respond. (These nutlets were kept and subsequently tested again in June 1988. At that time an additional 5.3 % did germinate.) Ronald Kelley (UCD) and Robert Ornduff (UCB) also report high germination in A. grandiflora (personal communications 1987). It appears that nutlet viability and germination will not pose significant barriers to the establishment and growth of new populations if the quality of nutlets is similar to that produced by Davis plants.

Seedling growth and development. Root hairs were produced on radicles within 24 hours of emergence from the pericarp. Elongation of the root averaged 5.4 mm/day during the first three days following germination, with a maximum rate of 7.0 mm/day (n = 10 germinules). The bifid cotyledons turned deep green after 2 days of exposure to the artificial growth light. The pericarp was usually shed from the tips of cotyledons within 4-5 days past germination, but in some seedlings it remained firmly attached and prevented the cotyledons from spreading out on both sides of the apex. As a

result, a "bird cage" of cotyledons was observed in 16% of the seedlings. The growing apex and expanding leaf primordia became entangled in the cage, stunting the growth of the seedling. The expansion of cotyledons and true leaves was significantly inhibited by the persistent pericarp (Table 1). Although not a source of mortality in and of itself, this inhibition of initial growth and the reduction in photosynthetic surface area would be a significant disadvantage within a dense community of rapidly growing grass and forb seedlings.

Table 1. Effect of the unshed pericarp on the growth of A. grandiflora seedlings (20 days old). Mean values (± S.D.) in a column followed by the same letter are not significantly different at P<0.025 (ANOVA).

type	cotyledon length (cm)	# of leaves	sum of leaf lengths (cm)
normal seedling (n= 41)	19.67 ± 4.71^{a}	4.6 ± 0.9^{c}	66.0 ± 23.6^{d}
caged seedling (n=9)	11.44 ± 4.22^{b}	4.2 ± 1.1^{c}	45.2 ± 19.3^{e}

The seedlings grew rapidly after transfer to peat pots. Mortality was low during this time, with 57 of the 60 germinules surviving to the pre-flowering stage (Table 2). Unlike plants in the field, the Mills plants were elongated and somewhat decumbent, rather than erect. They appeared to produce a greater number of leaves and branches as well (see below).

Of the original 60 germinules, 50 had survived and flowered 57 days after the end of the germination period. The cause of plant mortality during the transition to reproduction was not always apparent, but a virus-like infection was responsible in at least 3 individuals. Reproductive survivorship was high (83% = 50/60 X 100), as might be expected for cultivated annuals. What was not expected was the 40/10 (4/1) pin/thrum ratio in these plants. Ornduff (1976) found that the ratio varied between 1/1 and 2/1 at Site 300, while Taylor (1987) found 1.2/1 and 0.75/1. UC plants had an

approximate ratio of 1.5/1. This raises the possibility that pin genotypes may be differentially produced by the Davis population. Transfer of the 18 plants (M-UC) to UC reduced the 4/1 ratio to 2.9/1 among plants remaining in the Mills garden.

Nutlet production was apparent on some plants within 2 weeks of floral anthesis. Only 20 out of the 50 M and M-UC plants had produced nutlets by the time of senescence. Possibly, the available pollinators did not achieve the necessary redistribution of pollen. A hard, rather unseasonal rainfall occurred after anthesis in mid-April and may have inhibited pollination. Pin and thrum individuals did not significantly differ in their production of flowers (means of 100 vs. 112 flowers/plant, respectively) or nutlets (means of 46 vs. 52 nutlets/plant, respectively from M plants). Differences in pin/thrum fecundity were reported for plants in situ by Ornduff (1976) and Taylor (1987), but the latter also pointed out that this was not true during 1987.

Of the 18 flowering plants moved to UC, 12 died without setting fruit and only 6 produced nutlets. These six M-UC (all pins) were vegetatively the same size as pin M plants, but tended to produce fewer flowers (means of 77 vs. 100 flowers/plant) and nutlets (means of 13 and 46 nutlets/plant). Compared to Oakland, the cooler, more humid conditions of UC's Strawberry Canyon garden were somewhat less favorable for plant survival and nutlet production (see below). Statistically, however, there was no difference in nutlet production between M and M-UC plants and so all subsequent analyses will simply lump them together as "Mills" plants (grown two per pot). Of the total 75 nutlets sown in January, 732 nutlets were produced (Table 2).

Nutlet and ovule production - Linear correlations between nutlet or ovule output per plant and various measures of plant size were made using data from UC plants, Mills plants (M and M-UC) and Mills - UC plants combined (Table 3). The best-fit relationships (those with the largest sample size (n) and highest correlation coefficients (r)) are shown in Figures 2, 3 and 4 (these figures combine data from UC and Mills plants). Larger individuals (as measured by shoot weight or shoot length) produced inflorescences with greater total length and higher reproductive potential

Table 2. Demographics and phenology of the cultivated Mills population of Amsinckia grandiflora, 1988. Data from M and M-UC plants are combined (see text).

event/feature	value	observation date
initial number of nutlets	75	1/15
number of germinules	60	1/25
number of transplanted seedlings	59	1/25
number of established plants	57	2/18
number of flowering plants	49	3/23
number of plants producing >1 nutlet	20	5/5
number of nutlets produced (total)	732	5/5

(i.e. ovule output). There was a strong linear relationship between nutlet output and shoot length, shoot weight and total inflorescence length, meaning that larger plants, however assessed, were more fecund than smaller plants. The least amount of variability was found in the total inflorescence length-nutlet output relationship. The maximum number of nutlets from a single Mills plant was 110, with an average-sized plant (height = 39 cm, total inflorescence length = 36 cm) producing approximately 37 nutlets (Table 4). The maximum number of ovules produced by a single Mills plant was 720.

The strength of these relationships indicates that useful estimates of in situ nutlet production can be obtained using non-destructive measurements of plant height or total inflorescence length. It is recommended, however, that additional data be obtained from field plants at Site 300 or at the reintroduction site in order to increase the accuracy of the equations and their estimates. For example, Taylor (1987) found that plant height at Site 300 averaged 28.1 cm (1986) and 21.4 cm (1987) and produced an average of 6.0 and 3.6 nutlets/plant, respectively. Using Taylor's field heights and the greenhouse-derived relationship (UC+Mills) shown in Table 3 and

Figure 3, the estimate of nutlet production in situ would be 10.6 and 0 nutlets/plant. Although these estimates compare reasonably well with Taylor's measured values, adding the smaller but reproductive field plants to the analysis would increase accuracy at the lower threshold of reproductive size.

The difference between potential reproductive output (number of ovules per plant) and actual reproductive output (number of nutlets per plant) is a measure of reproductive efficiency - the ability of a plant to transform its ovules into seeds. On the average for Mills and UC plants, only 12.5% of the total ovules produced were converted into seeds, with a maximum of 21.6% (Figure 5). This compares with 30% conversion in situ (Ornduff 1976) and 40% after hand pollination in the greenhouse (Weller and Ornduff 1977). Other endangered plants can be more efficient (Swallenia alexandrae (36%), Erysimum capitatum var. angustatum (49%), Oenothera avita ssp. eurekensis (65%)), but some are not (Astragalus lentiginosus var. micans (12%)) (Pavlik and Barbour 1985, Pavlik et. al. 1988).

These results confirm that low seed production per individual severely restricts population growth of Amsinckia grandiflora. Ornduff (1976) reported that other annual Amsinckia species produce more nutlets than Amsinckia grandiflora. The abundant and widespread A. tessellata and A. intermedia are homostylous and capable of producing 2-3X as many nutlets per flower as A. grandiflora. Large individuals of the weedy A. hispida produce more than 1000 nutlets each, with averages in the hundreds (Connor 1965). Other endangered or threatened plant species can produce hundreds or thousands of seeds per individual. At Eureka Dunes, Pavlik (1987) reported that on average, the endangered Oenothera avita ssp. eurekensis (an herbaceous perennial) can produce 36,000 seeds under favorable field conditions and 7,000 when vegetative growth is restricted by poor conditions. The largest Oenothera individuals produced as many as 65,000 seeds. At Antioch Dunes National Wildlife Refuge, an average E. capitatum var. angustatum (an annual or biennial) produces 2,800 seeds (Pavlik et. al 1988). Greenhouse-grown A. grandiflora produce fewer seeds by one or two orders of magnitude and field-grown plants by three orders, compared to these taxa.

Table 3. Linear correlations between various measures of plant size and nutlet output, ovule output or reproductive efficiency per individual Amsinckia grandiflora. UC = plants grown in mass by UC Botanical Garden. Mills = plants grown in pots at Mills (M) or at UC (M-UC). **Bold type** indicates the relationship shown in Figures 2-5 and recommended for use in subsequent studies. ns = not significant, Σ inflor lgth = sum of the lengths of all inflorescences, repro eff = reproductive efficiency

plants	n	X	Y	slope	intercept	r	P
UC	10	shoot weight (g)	#nutlets	16.885	19.200	0.48	ns
Mills	20	" "	" "	23.006	-13.362	0.65	<0.01
UC+Mills	**30**	" "	" "	**15.639**	**8.637**	**0.52**	**<0.01**
UC	10	shoot length (cm)	#nutlets	1.230	-18.545	0.56	ns
Mills	20	" "	" "	2.511	-60.766	0.78	<0.01
UC+Mills	**30**	" "	" "	**2.014**	**-45.971**	**0.71**	**<0.01**
UC	9	Σ inflor lgth (cm)	# nutlets	0.914	12.991	0.65	<0.05
Mills	20	" "	" "	1.199	-6.192	0.88	<0.01
UC+Mills	**29**	" "	" "	**1.129**	**-0.301**	**0.84**	**<0.01**
UC+ Mills	30	shoot weight (gm)	#ovules	15.639	8.637	0.52	<0.01
UC+Mills	30	shoot length (cm)	#ovules	9.406	-33.469	0.53	<0.01
UC+Mills	**29**	**Σ inflor lgth (cm)**	**#ovules**	**7.708**	**102.332**	**0.93**	**<0.01**
UC+Mills	**29**	**Σ inflor lgth (cm)**	**repro eff**	**0.001**	**0.055**	**0.44**	**<0.05**

Comparison of culture methods - Plants grown at low density in pots (Mills) had more shoot biomass (Table 4) and a greater number of branches than did plants grown in a single lot at high density (UC). Shoots of UC plants were significantly longer and narrower (due to less branching) and more closely resembled shoots at Site 300 where competition with annual grasses would produce the same growth form. Although Mills and UC produced statistically similar numbers of nutlets, the larger Mills plants tended to produce more ovules and would, therefore, have greater reproductive potential. UC plants, however, were more efficient in converting ovules into nutlets, perhaps because the aggregated population was showier and attracted more pollinators than did the low density Mills plants.

Another method of cultivating Amsinckia grandiflora was used in the experimental garden at UC Davis (R. Kelly, personal communication 1988). After solarization to destroy weed seeds, nutlets were sown into shallow furrows and covered. They received only occasional waterings and limited amounts of hand-tending. These individuals grew to be quite large (much larger than those at Mills or UC) and could produce approximately 250 nutlets each on the average. Possibly the warmer, less humid Davis climate was more favorable to growth than the Bay Area climate, but R. Kelley also believes that the lack of tap root restriction in the garden furrows was also an important factor. Under these culture conditions, A. grandiflora grew largest and achieved the highest output of nutlets per individual. From the standpoint of propagation, the Davis method is more productive and less labor-intensive than the Mills or UC methods.

With respect to propagation and reintroduction efforts, it is important to favor vegetative growth and the production of large plants while increasing reproductive efficiency. This could be accomplished by sowing nutlets directly into deep soil, controlling competition from annual grasses (controlled burning, grass herbicide, hand weeding) and positioning colonies of effective pollinators (e.g. Apis mellifera, Ornduff 1976) near the population.

Quality of nutlets produced - It was possible to distinguish two distinctive size categories of nutlets from the Mills and UC populations. Large and small nutlets could

Table 4. Comparison of plants grown under different culture conditions. UC plants were grown at high density by UC Botanical Garden while Mills plants (M and M-UC combined) were grown at low density by Mills Botanical Garden. Mean values (± SD) for Mills and UC in a row followed by the same letter are not significantly different (ANOVA, $P<0.05$).

	Mills (n=20)	UC (n=10)	Mills + UC (n=30)
shoot weight (g)			
mean	2.17 ± 0.91[a]	1.15 ± 0.39[b]	1.83 ± 0.93
maximum	3.59	2.16	3.59
number of branches			
mean	7.9 + 2.6[c]	3.5 + 0.9[d]	5.7 + 2.3
maximum	12	7	9
shoot length (cm)			
mean	38.8 ± 10.0[e]	46.4 ± 6.2[f]	41.3 ± 9.8
maximum	63.0	55.0	63.0
Σ inflorescence length (cm)			
mean	35.7 ± 23.6[g]	27.0 ± 10.2[g]	33.0 ± 21.1
maximum	87.0	49.5	87.0
# nutlets/plant			
mean	36.6 ± 33.1[h]	38.6 ± 14.5[h]	37.3 ± 27.9
maximum	110	71	110
# ovules/plant			
mean	389.2 ± 189.7[i]	287.6 ± 86.7[i]	355.3 ± 172.5
maximum	720	504	720
repro efficiency			
mean	0.076 ± 0.083[j]	0.137 ± 0.041[j]	0.096 ± 0.057
maximum	0.163	0.216	0.216

be found on nearly every individual examined, but Mills plants produced a significantly greater proportion of small nutlets (84.4 ± 13.5 % of the total nutlets produced) than did UC plants (14.4 ± 8.7%). On the average, large nutlets weighed over 3 mg and small ones less than 2.0 mg (Table 5). The original suspicion was that the small nutlets did

not contain viable embryos or sufficient amounts of stored food to allow germination and seedling survival. This was not the case because subsequent trials showed that only small UC and small Mills nutlets tended to germinate without special treatment (Table 5) and grow into seedlings. Large UC and Mills seeds probably have an after-ripening requirement that the small seeds do not. If so (this will be tested by germination trials in late fall 1988), the co-occurence of an additional dormancy mechanism with the large nutlet morph indicates another reproductive polymorphism in Amsinckia grandiflora. The implication is that under a more competitive growth regime (in this case, intraspecific competition induced by the high density UC culture method), Amsinckia produces large nutlets (presumably with more resources per nutlet) having restrictive germination requirements. Under a less competitive growth regime (in this case the low density Mills culture) Amsinckia produces more ovules and smaller nutlets (less resources per nutlet) having more liberal germination requirements. Differences in nutlet resources may result in differences in the competitive ability of seedling that match the competitive regime of the habitat. This is an exciting possiblity that requires more sophisticated experimentation before solid conclusions are drawn. It does demonstrate that seeds produced at Mills are of good quality despite their smaller size and that these culture methods are effective in increasing seed supply.

Table 5. Weight and germination of nutlets produced in 1988 using Mills and UC culture methods. Segregation of nutlets into large and small categories was done visually. Mean values (± SD) within a row followed by the same letter are not statistically different (ANOVA, $P<0.01$).

	Mills		UC	
	large	small	large	small
nutlet weight (mg)	3.00 ± 0.52^a	1.33 ± 0.56^b	3.93 ± 0.36^c	1.84 ± 0.48^b
germination (%)	45	25	0	40

Conclusions and Management Recommendations

1) The methods used to cultivate Amsinckia grandiflora at Mills College were effective in amplifying the original population from 75 to 732 nutlets. Those nutlets were of a generally small size, but at least 25% exhibited the ability to germinate within two months of production. From a standpoint of efficiency, however, only 12% of the available ovules were converted into seeds. This could have been due to pollination limitations, possibly linked to the number of effective pollinators in the vicinity of the Mills Botanical Garden. This method is recommended when nutlets are in short supply and the population of effective pollinators can be enhanced.

2) The methods used to cultivate Amsinckia grandiflora at UC Berkeley Botanical Garden were also effective with respect to nutlet production. The nutlets produced were larger than Mills nutlets, but they also possessed an additional dormancy mechanism that prevented an assessment of germination ability. It appears that although the high density culture method reduced plant size and reproductive potential, reproductive efficiency was enhanced relative to the low density Mills method. This method is recommended when nutlets are not in short supply (although data on establishment efficiency are lacking) and when pollinator populations may be limited.

3) From a standpoint of increasing the number of nutlets available for establishing new populations, the cultivation of Amsinckia grandiflora at UC Davis is the best way to amplify the existing supply of nutlets. This method is recommended when nutlets are not in short supply (although data on establishment efficiency are lacking).

4) Germination percentage of large nutlets (6 months post-production) was high and probably does not present a significant barrier to the establishment and growth of populations.

5) A number of other phenomena observed in this study could, however, inhibit population growth. These included 1) "caging" and subsequent inhibition of seedling growth by attached pericarps, 2) a very eccentric pin/thrum ratio of plants germinated from Davis nutlets, 3) high mortality in the stage between flowering and nutlet production, 4) inefficient conversion of ovules into seeds, and 5) the possible production of different kinds of nutlets with different dormancy mechanisms, resource reserves and competitive abilities. Whether or not these phenomena are important in situ will have to be tested from detailed demographic studies on new satellite populations.

6) There was a strong linear relationship between nutlet output and shoot length, shoot weight and total inflorescence length, meaning that larger plants, however assessed, were more fecund than smaller plants. The least amount of variability was found in the total inflorescence length-nutlet output relationship. The strength of these relationships indicates that useful estimates of in situ nutlet production can be obtained using non-destructive measurements of plant height or total inflorescence length. It is recommended, however, that additional data be obtained from field plants at Site 300 or at the reintroduction site in order to increase the accuracy of the equations and their estimates.

7) Since nutlets can be effectively obtained by cultivation, it is recommended that a large quantity (1500-1800) be obtained from a variety of sources (UC Berkeley, Mills and UC Davis) and used to establish a new population of Amsinckia grandiflora within its historic

range. The results of this study indicate that during the establishment of the new population, it will be important to favor vegetative growth and the production of large plants while increasing reproductive efficiency. This could be accomplished by sowing nutlets directly into deep soil, controlling competition from annual grasses (controlled burning, grass herbicide, hand weeding) and selecting a site with good potential for supporting populations of effective pollinators.

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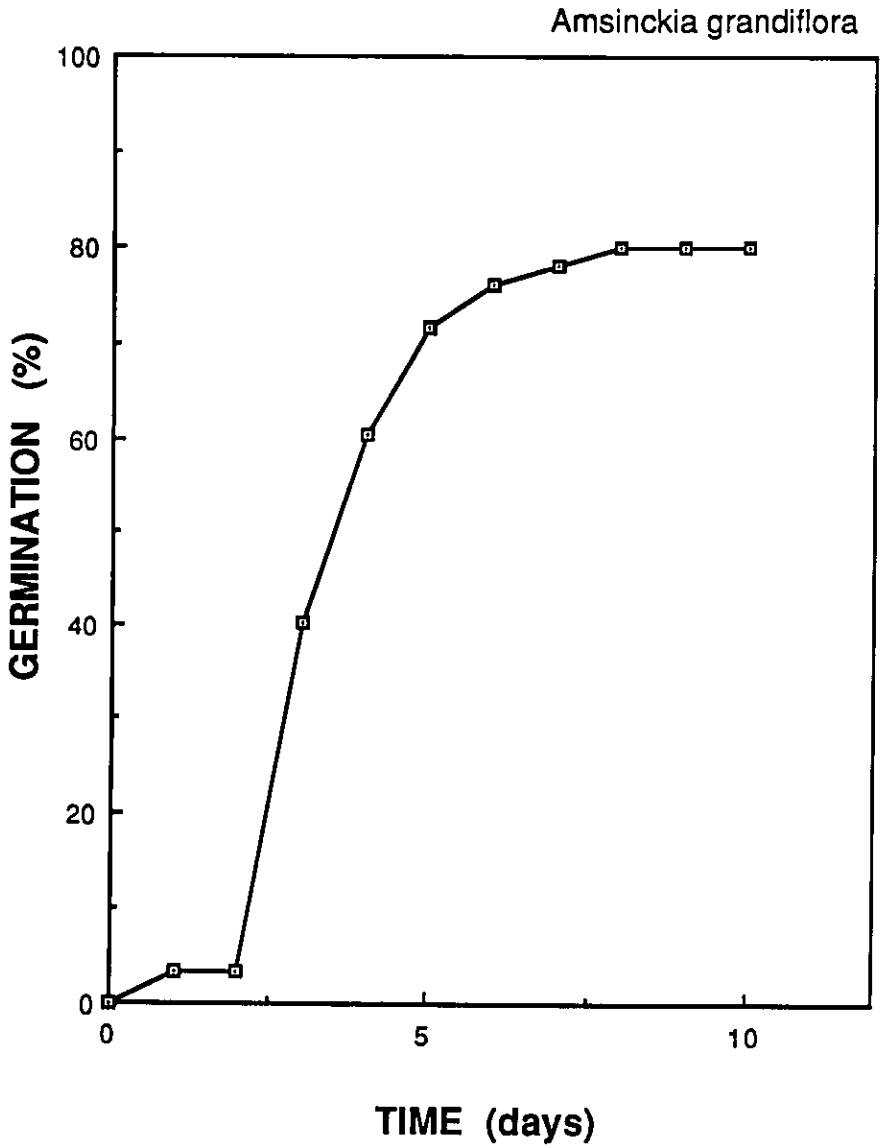

Figure 1. Laboratory germination of Amsinckia grandiflora nutlets. The curve is a mean of three replicates. See text for details.

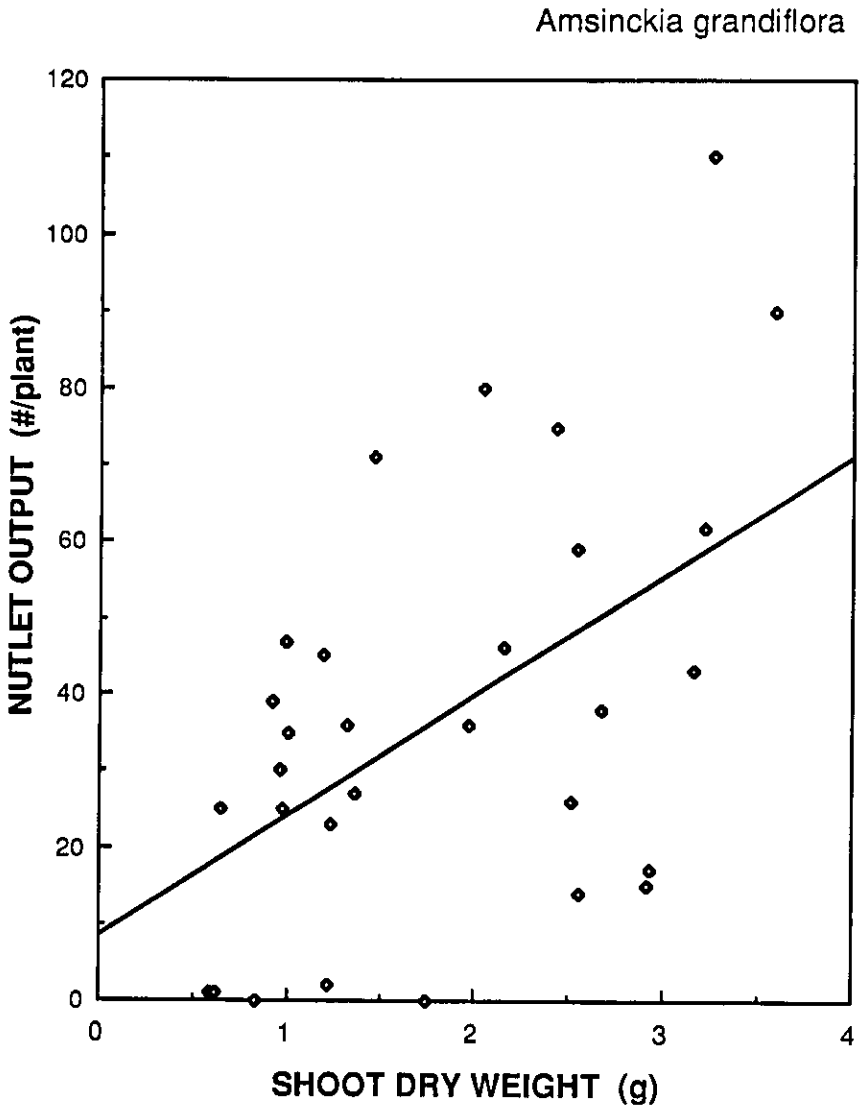

Figure 2. Nutlet output (number of filled, undamaged nutlets per plant) as a function of shoot dry weight in garden-grown plants of Amsinckia grandiflora. Data from UC and Mills plants combined. See text for equation of the line.

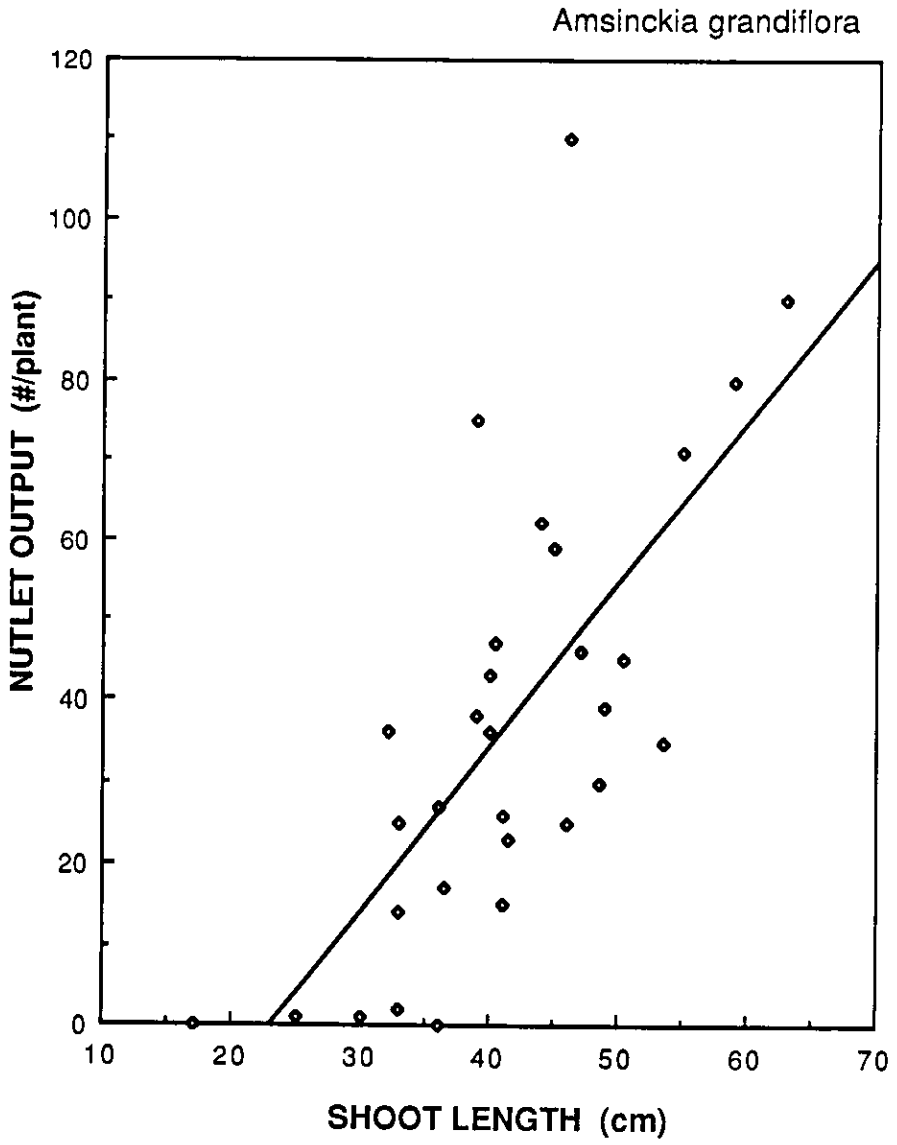

Figure 3. Nutlet output as a function of shoot length in garden-grown plants of Amsinckia grandiflora. Data from UC and Mills plants combined. See Table 3 for equation of the line.

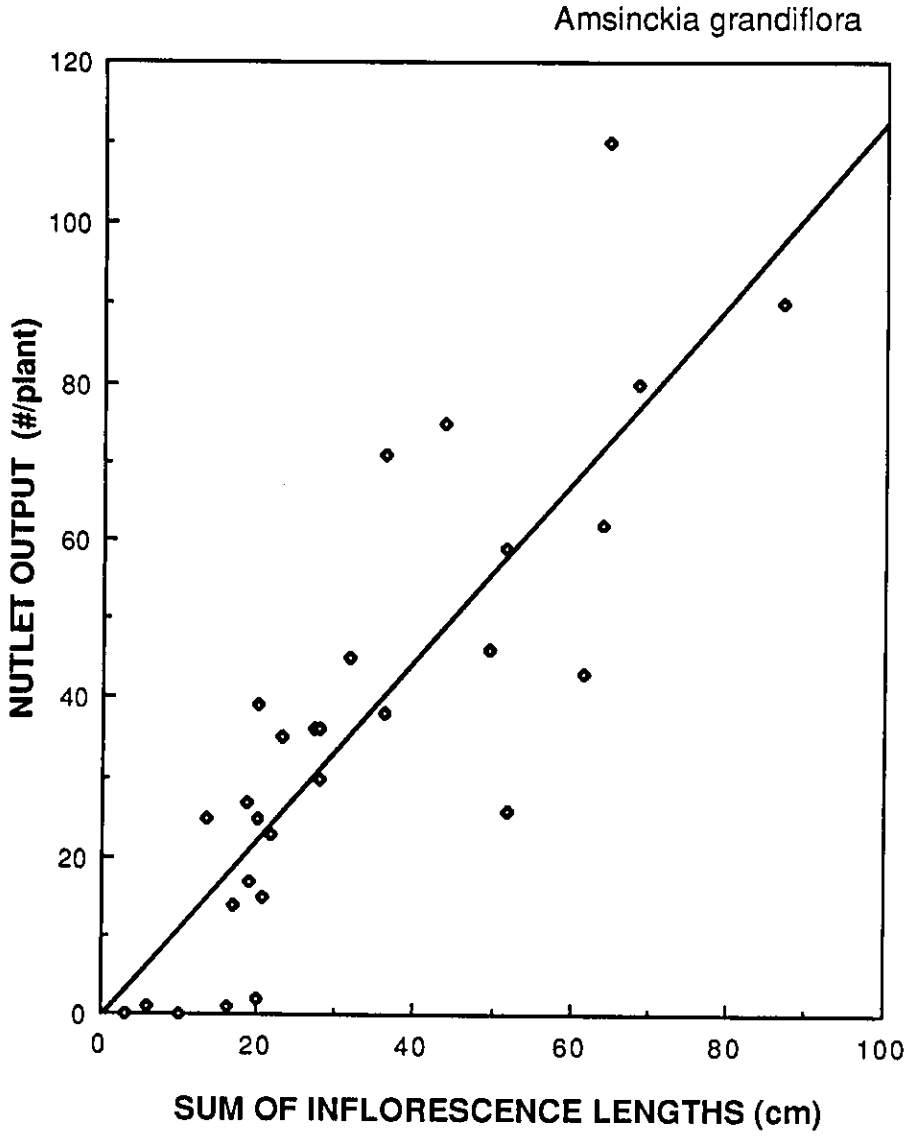

Figure 4. Nutlet output as a function of the total length of all inflorescences on an individual in garden-grown plants of Amsinckia grandiflora. Data from UC and Mills plants combined. See Table 3 for equation of the line.

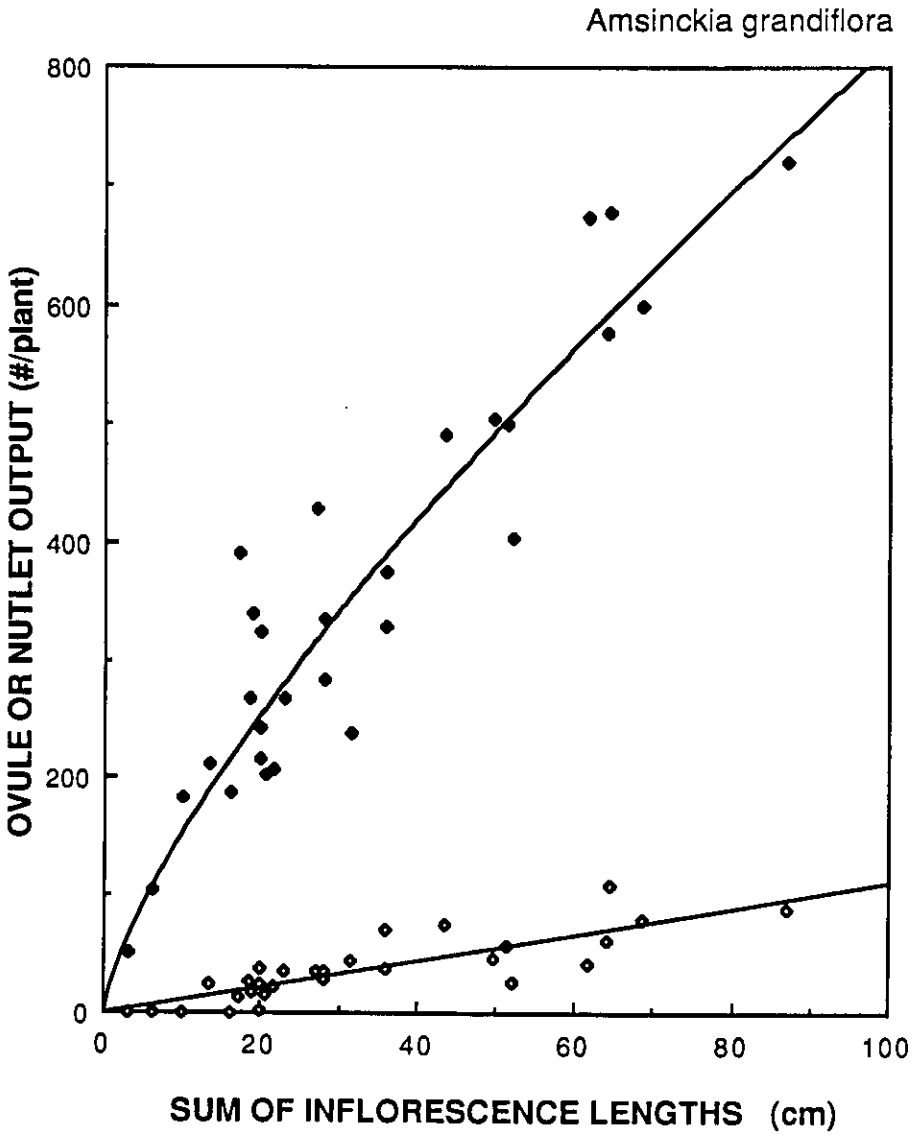

Figure 5. Comparison of potential reproductive output (number of ovules per plant, closed symbols) to actual reproductive output (number of nutlets per plant, open) in garden-grown plants of Amsinckia grandiflora. See Table 3 for equations of the lines.

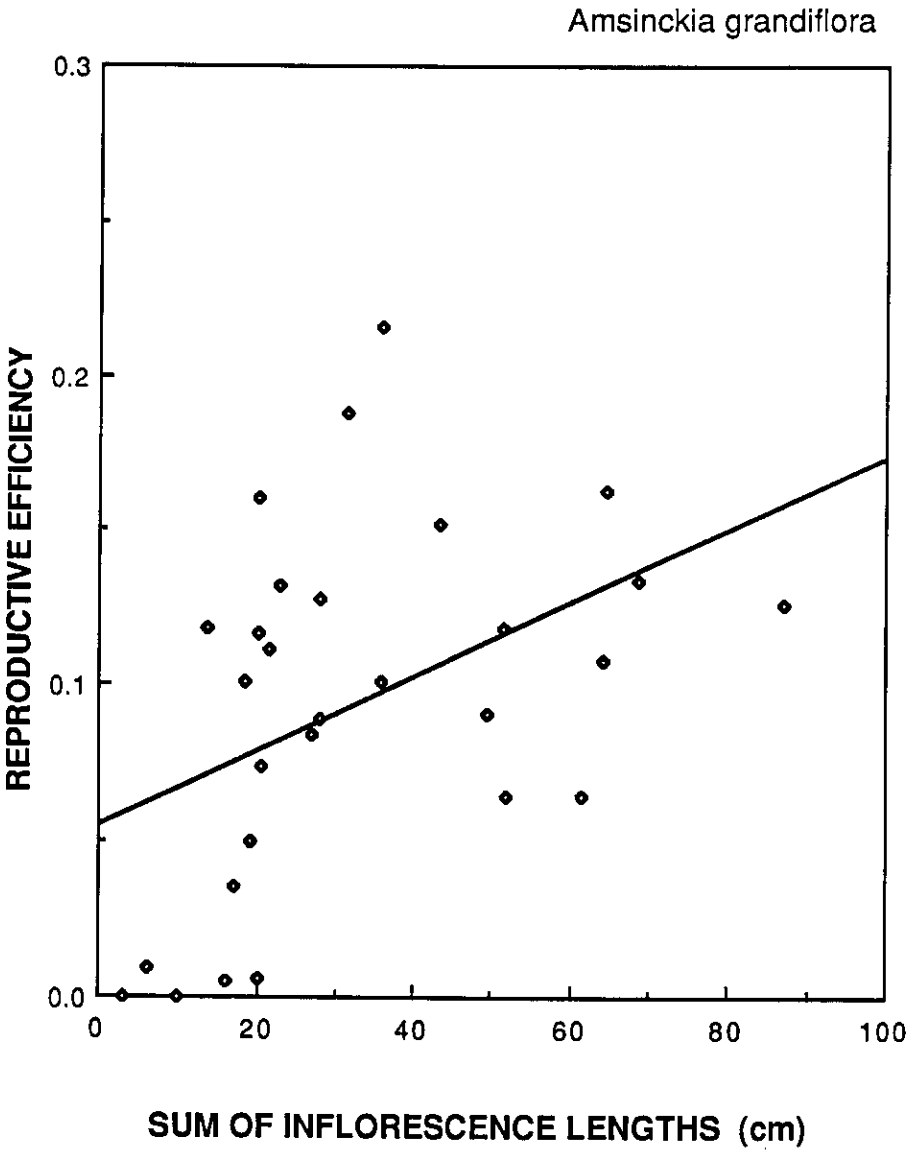

Figure 6. Reproductive efficiency (ratio of ovules to nutlets per plant) as a function of plant size in garden-grown plants of Amsinckia grandiflora. See Table 3 for equation of the line.

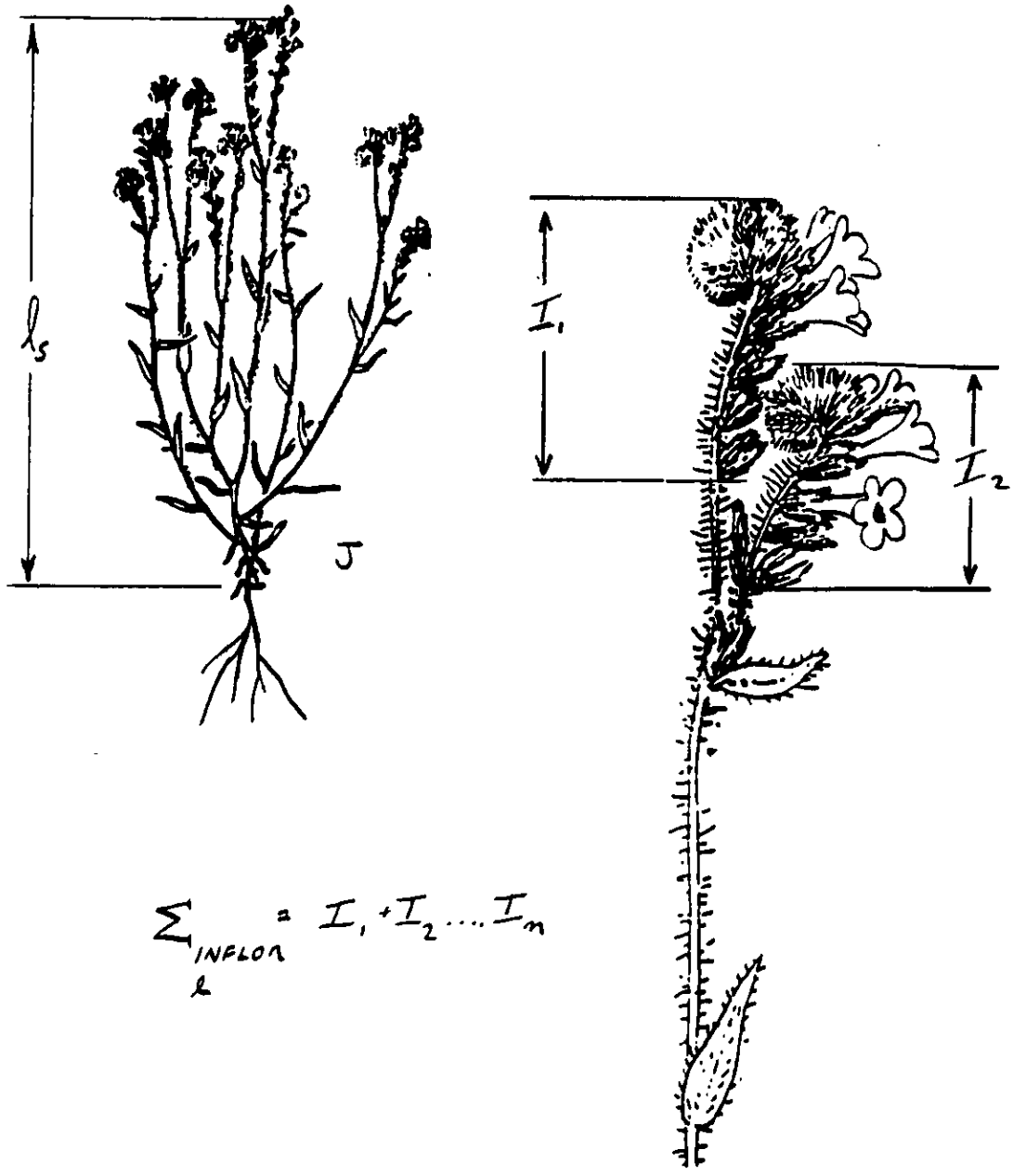

Figure 7. Shoot length (ls) and inflorescence length (I) measurements made on Amsinckia grandiflora. Formula shown is for the sum of all infloresence lengths (n) from a single plant. Drawing by J. Janish, from Illustrated Flora of the Pacific States, by L. Abrams.