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Significance of Bulb Polarity in Survival of Transplanted Mitigation Bulbs

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Abstract.—Our experimental design was formulated to determine whether or not bulb polarity (orientation) at the time of replanting of bulbs to salvage plants of *Calochortus weedii* A. W. Wood (Liliaceae) or Weed's Mariposa Lily affected the success of the mitigation transplant effort. Polarity of bulbs at planting clearly did influence subsequent growth, most notably in the tip-down (D) treatment. Among these bulbs, 75% failed to emerge from dormancy and only four (20%) actually set mature fruit. This was in sharp contrast to the other three treatments where 100% of the bulbs successfully emerged in this season and between 80% (S) and 95% (UG and UN) set mature fruit. The data from this study do indicate that: 1) bulb planting orientation does influence survival and growth, and 2) proper bulb planting polarity (orientation) should be an important consideration in any transplantation of this or any sensitive bulb producing plant species for mitigation purposes.

In general, when planted, bulb polarity is important and bulbs should be planted with the apex up and the root base down (Hitchmough and Fieldhouse 2003). However, if bulbs are inadvertently planted sideways or upside-down how significant is that to bulb survival and/or subsequent reproductive productivity? Such knowledge becomes especially important when the manipulated bulbiferous plant is a rare and endangered species and the bulbs are being salvaged and transplanted as part of a mitigation process. It is in this context that the current study was conceived. This study was initiated at the request of the U. S. Fish and Wildlife Service Agency. Specifically, the question under consideration is: "In Weed's Mariposa Lily, *Calochortus weedii* var. *intermedius*, does the tip orientation (polarity) of bulbs have a significant effect on subsequent survival and reproduction?" Results are intended to assist future mitigation efforts when applied to this and perhaps other rare and endangered bulbiferous species.

Calochortus weedii var. *intermedius* (hereafter - CWI) is a single-leaved herbaceous perennial that develops from a small bulb (Fig. 1). Bulbs were defined as the swollen basal portion after the thinner elongated portion, made up of the dried senescent portions of the inflorescence and associated leaf bases, was removed. It is distinguished from the three other varieties by anther shape, flower color, and petal shape (Ownbey 1940; Wiggins 1980; Hickman 1993; Fielder 1996). It is included in the CNPS Inventory of Rare and Endangered Plants on list 1B.2 (*rare, threatened, or endangered in CA and elsewhere*) (Tibor 2001; California Native Plant Society 2013). CWI bulbs generally follow the *Calochortus* life history or pattern of development described by Fiedler (1987) in her study of five primarily Central California species. After fall rains, the small bulbs emerge from late summer/fall dormancy, producing the single basal leaf. Following several months of leaf elongation, an inflorescence stalk develops. Flowers

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Fig. 1. Typical bulb of CWI. Bulb includes area from left side of figure to the 3 cm position on the ruler.

appear in mid-spring and by July-August fruit capsules mature, seeds disperse, flower stalks dry down, and the bulbs once again become dormant.

Materials and Methods

CWI bulbs used in this study originated in Los Trancos Canyon, Orange County, California just inland from northern portions of Crystal Cove State Park, at the east end of the San Joaquin Hills. To examine if orientation had any affect on bulb transplantation success, we used four bulb planting orientation treatments encoded UN, UG, S, and D as follows: 1) UN, tip (bulb apex) up, in native soil (used as a control for comparison to treatments in which the soil was a standard greenhouse mix, see below for details); 2) (UG) tip up, in greenhouse soil; 3) (S) tip to the side; i.e., parallel to the soil surface, in greenhouse soil; and 4) (D) tip down, in greenhouse soil. The two tip-up groups (UN and UG) served as controls. Twenty bulbs were selected for each treatment.

Square plastic pots (2.6 liter) were utilized and bulbs were planted in the center of the pot at a 5 cm depth. Native soil for the UN treatment was provided by LSA Associates, Inc., Irvine, California, (hereafter LSA). All other treatment pots were filled with a greenhouse soil mix, which included one part native soil (for possible mycorrhizal considerations) to three parts standard greenhouse soil by volume. This latter greenhouse soil was a mix of an organic fraction (50%) that included peat moss (6 parts by volume) and forest humus (9 parts by volume) plus an inorganic fraction (50%) that included washed plaster sand (6 parts by volume) and pumice (9 parts by volume). Sierrablen (Everiss International) time-released fertilizer (NPK 18N:7P:10K + Fe) was added at the rate of 4oz/10 gallons soil mix and dolomite (Ca & $MgCo_3$) at 5oz/10 gallons of soil mix.

Pots were randomly placed on outdoor benches in the California State University, Fullerton (CSUF) Biology Greenhouse Complex where they were subject to natural environmental temperatures (Fig. 2). The pots on the bench were surrounded by cement blocks in order to provide the outer pots with a heat load similar to that experienced by other pots on the bench. During both two-year studies (2003-05 and 2005-07) supplemental water was provided when the pot soil was dry to a 2.5 cm depth. All watering (natural or artificial) had ceased by mid-July when fruit capsules were ripening, the flower stalks were withering, and the bulbs had entered summer dormancy.

Fig. 2. Benches used in study. Located in the California State University, Fullerton, California, Biology Greenhouse Complex.

Experiment 1, using wild-collected bulbs (2003-05).—Two batches of newly-dug CWI bulbs were provided by LSA, one each on October 16 and 18, 2003. To minimize possible bulb size effects, larger bulbs weighing at least 3g, as determined using a Mettler AE163 balance, were selected and placed in numbered coin envelopes (No. 1 coin envelopes – 2.25 X 3.5 in.) forming a pool of 160 bulbs for potential study. Bulbs were selected for the various treatments with the use of a Random Number Table (Zar 1974). Selected bulbs ranged in weight from 3.8 to 7.9g with a mean weight of 4.8g. Planting occurred on October 19, 2003 and the resulting plants were followed through 2005 until the bulbs were harvested on September 30, 2005. Harvested bulbs were subsequently individually weighed and returned to LSA.

Experiment 2, using propagated bulbs (2005-07).—Bulbs for the second two-year study were descended from the original field collection of October 2003, but these bulbs had been propagated by the Tree of Life Nursery in San Juan Capistrano, California and were then provided to us by LSA in October 2005. Individual bulbs were selected for this study using the same methods described for the first two-year study; however, these bulbs were significantly smaller ($t=222.0$, $df=79$, $P>0.001$), so a minimum weight to be included was established at 1.5g. Selected bulbs ranged in weight from 1.7g to 5.6g with a mean weight of 2.6g. Planting of these bulbs occurred on October 21, 2005 and the resulting plants were followed through 2007 until the bulbs were harvested on September 28, 2007, individually weighed. All recovered bulbs were returned to LSA upon completion of the study and the submission of the final report.

Where appropriate, data were analyzed using a Student's t-test, analysis of variance (ANOVA) in JMP, version 5.0 or an analysis of covariance (ANCOVA) or a logistical regression in JMP version 5.0. Homogeneity of variance and normality were examined by looking at residuals and normal probability plots of residuals.

Results

Maximum and minimum temperatures were recorded during both two-year studies (2003-2005 and 2005-2007). There were six weeks with average temperatures at or above 30°C in

2003-04, two such weeks during 2004-05, seven such weeks in 2005-06, and four such weeks in 2006-07. However, overall the weekly pattern of temperatures was similar over the four-year study.

Experiment 1, Year 1.—During the 2003-04 growing season, all twenty newly planted bulbs in the UN, UG, and S treatments ultimately produced leaves; however, only five of the tip-down (D) did so. One hundred percent leaf emergence occurred two weeks earlier (Week 14, mid-January) in the native-soil control bulbs (UN) than in the greenhouse-soil controls (UG) and side-planted (S) bulbs (Week 16, early February). Maximum emergence (25%) of D (tip-down) bulbs was registered even later in Week 20 (early March).

Experiment 1, Year 2.—In the 2004-05 growing season, 100% of the previously side planted bulbs produced leaves, whereas 95%, 90%, and 65% of the UG, UN, and D bulbs, respectively, did so. More than 85% of the UN, UG, and S bulbs had produced a leaf by the end of the Week 11, whereas only 10% of the D bulbs had done so by that same time. (Note that leaf emergence proceeded faster during the 2004-05 season than it did during the 2003-04 season).

Experiment 2, Year 1.—During the 2005-06 growing season, eighteen of the newly planted S and UG bulbs, as well as 19 of the UN and only 9 of the D bulbs ultimately produced leaves. Furthermore, leaf emergence did not proceed at the same rate in the treatments during that year. Leaf emergence occurred in UN (bulbs in native soil) plants much more rapidly than any of the other treatments.

Experiment 2, Year 2.—In the 2006-07 growing season, many fewer previously planted bulbs experienced leaf emergence. Fourteen of the UN, 15 of the UG, 18 of the S, and only 8 of the D bubs produced a leaf.

Inflorescence initiation.—Once the basal leaf has reached maturity and the plant begins to put forth an inflorescence, the basal leaf rapidly begins to wither away and is replaced by an inflorescence stalk. In the 2003-04 growing season, development of inflorescence stalks was first noted in the two tip-up control groups (UN and UG) during the second week of February (Week 16). Side-planted bulbs (S series) began exhibiting developing flower stalks two weeks later (Week 18, late February), followed two weeks later (Week 20, early March), in the tip-down group (D treatment). Development of inflorescence stalks during the 2004-05 growing season was first noted in the two tip-up control groups (UN and UG) in the last week of January (Week 16) during the 2004-05 growing season. Side-planted bulbs (S) began exhibiting developing flower stalks in that same week (Week 16), followed two weeks later (Week 18, early February) by inflorescence development in the tip-down group (D).

During the 2005-06 growing season, development of inflorescence stalks in the newly planted bulbs was first noted in the two tip-up control groups (UN and UG) during the second week of January (Week 12). Side-planted bulbs (S) began exhibiting developing flower stalks during that same week (Week 12), followed six weeks later (Week 18, early February) by inflorescence development in the tip-down group (D).

Development of inflorescence stalks during the 2006-07 growing season was first noted in the previously planted tip-up control group, grown in native soil (UN), during the second week of January (Week 14). The other tip-up control group (UG), began to develop inflorescences during the first week in February (Week 17). Side-planted bulbs (S) began exhibiting developing flower stalks during the third week of February (Week 19), followed three weeks later (Week 22, second week in March) when the first instances of inflorescence development appeared in the tip-down group (D).

When mean times of inflorescence initiation during the 2003-04 growing season are considered by treatment, values of the UN, UG, and S group are approximately equivalent (19.7, 20.2, and 20.4 weeks, respectively) with the D group average differing at 22.5 weeks after planting.

These variations were not statistically different. An analysis (ANOVA) of the data for the 2004-05 growing season also showed no significant differences among all four treatments.

Not all plants formed inflorescences during either of these first two seasons. In each group at least one plant remained in the vegetative state with the basal leaf rapidly withering. These plants were categorized as "dead or dormant" (d/d). During the 2003-04 growing season in the S treatment, three plants out of 20 failed to form inflorescences, whereas in the UN and UG controls, it was one plant out of 20 and in the D treatment, of the five plants that had a basal leaf, only four formed inflorescences. During the 2004-05 growing season only 7 of the 20 UN bulbs planted in 2003 produced an inflorescence, whereas 10 of the S bulbs, 12 of the D bulbs, and 13 of the UG bulbs did so. Similarly, during the second two-year study (2005-06 and 2006-07) not all plants formed inflorescences during either of these second two seasons. In each group at least one plant remained in the vegetative state with the basal leaf rapidly withering. These plants were also categorized as "dead or dormant" (d/d). During the 2005-06 growing season, in the S treatment, three plants out of 17 that produced a basal leaf failed to go on to form inflorescences, whereas in both the UN and UG controls, four bulbs out of 19 and 18 respectively that produced a basal leaf failed to produce an inflorescence. In the D treatment, of the nine plants that had a basal leaf, only six formed inflorescences. Inflorescences were produced in fewer of the tip-down (D) bulbs than any of the other treatments. The other three treatments were all very similar.

During the 2006-07 growing season, only 7 of the 14 UN bulbs that had produced a basal leaf went on to produce an inflorescence, whereas 12 of 18 of the S bulbs, 5 of the 8 D bulbs, and 9 of the 15 UG bulbs that developed a basal leaf actually produced an inflorescence. Inflorescences were produced later in the tip-down (D) bulbs than in any of the other three treatments during this fourth year of study. However, side-planted (S) and those bulbs planted with the tip-up in greenhouse soil (UG) were not different from one another but they both different from the D and UN treatments. Those bulbs planted with the tip-up in native soil produced inflorescences earlier in the season than any of the other treatments.

Initiation of floral buds.—In contrast to the developmental aspects discussed above, floral bud formation occurred synchronously in all treatments during both years (2003-04 and 2004-05) of the first study: Week 24 (beginning in late March) of 2004 and during Weeks 26 and 27 (again beginning in March) of 2005. Floral bud formation also occurred synchronously in all treatments during both years (2005-06 and 2006-07) of the second two-year study, with 2006-07 showing the most spread. However, bud initiation for neither of these two years was notably different from bud formation during the first two years of study (2003-04 and 2004-05). Buds began to form in Weeks 25 to 27 (beginning in late March) of 2006 and during Weeks 23 to 27 (again beginning in March) of 2007.

Appearance of open flowers.—As with floral buds, open flowers appeared synchronously in all treatments during both growing seasons of the first two-year study. This occurred in mid-May or Weeks 29 to 30 of 2004 and in Weeks 30 to 32 of 2005. A similar pattern was observed during the second two-year study with flowers appearing in Weeks 29 to 32 of 2006 and in Weeks 29 to 31 of 2007.

Initiation of fruit set.—Again, this process was observed to be synchronous in all treatments during the first two-year study and was coincident with the appearance of open flowers during Week 30 and 32, mid-May of 2004 and during Weeks 33 to 35 of 2005. A similar pattern of fruit production was seen in the second two-year study with fruits appearing in Weeks 31 to 33 in both 2006 and 2007. However, fruit set began slightly earlier in 2003-04 than in the following years.

Table 1. Summary of data relative to growth in CWI for the four treatments and the overall average for all four treatments in the 2003-2004 study. Infl.=Inflorescence; Ave.=average; SD=standard deviation.

Trait	UN	SD	UG	SD	S	SD	D	SD	Overall average
Infl (N)	19		18		17		4		14.5
Ave. Infl. Height (mm)	745.5	51.4	851.9	58.2	817.5	54.6	818.3	50.7	808.3
Ave No. Branches	3.4	0.2	4.3	0.3	4.0	0.3	2.8	0.2	3.6
Ave No. Fruits	5.0	0.4	7.6	0.6	7.1	0.5	5.0	0.3	6.2

Number of mature fruit produced.—The four treatments fell into essentially two groups in terms of mean fruit production during the 2003-04 growing season (Table 1). The tip-down treatment (D) and the native-soil control plants (UN) were essentially equivalent, producing an average of 5.0 (D) and 5.1 (UN) mature capsules, respectively, per plant. Fruit production for the side-planted (S) and the greenhouse-soil controls (UG) was approximately 30% higher, with mean values of 7.1 and 7.7, respectively. An analysis of variance (ANOVA) showed significant differences among the four groups ($P<0.05$), with UN being significantly different from UG and S, but not from D. All other treatments were not significantly different from one another.

Fruit production during the 2004-05 season (Table 2) was only significant different between the two controls (UN and UG). Fruit production during the 2005-06 season showed some significant differences among the four treatments ($P<0.05$) using an analysis of variance (ANOVA), with only the tip-down (D) bulbs being significantly different from the side-planted (S) bulbs, but neither of those were significantly different from either of the controls (UN or UG planted bulbs). Fruit production during the 2006-07 season showed significant differences among the four treatments ($P<0.05$) using an analysis of variance (ANOVA), with the tip-down (D) bulbs being significantly different from the up-greenhouse bulbs (UG control), but neither of those was significantly different from either of the controls (UN or S planted bulbs).

Summaries of reproductive information.—The following tables summarize the data relative to reproduction for this species during the four growing seasons: 2003-04 (Table 1); 2004-05 (Table 2); 2005-06; (Table 3); and 2006-07; (Table 4). In nearly all cases (except inflorescences produced per plant in the down treatment in 2004-05 and the average number of fruits produced per inflorescence in the 2006-07), reproductive output as measured by inflorescence characteristics and fruit production was lower in the year following the initial planting season (2004-05 versus 2003-04 and also in 2006-07 versus the 2005-06 season). Reproductive fitness was severely limited by the tip-down orientation of bulb planting during all four seasons. Only four of the tip-down CWI bulbs produced flowers and fruits during the 2003-04 and 2004-05

Table 2. Summary of data relative to growth in CWI for the four treatments in the 2004-2005 study with percentage similarity to 2003-2004 for comparison.

Trait	UN	%	SD	UG	%	SD	S	%	SD	D	%	SD	Overall average
Infl (N)	6	32		8	44		10	59		4	100		6
Ave. Infl. Height (mm)	534.2	72	41.8	432.3	51	29	451.8	55	31.3	393.3	48	28.5	452.3
Ave No. Branches	2.2	65	0.1	1.0	16	0.1	1.9	27	0.1	1.5	30	0.1	1.8
Ave No. Fruits	2.7	43	0.2	1.3	17	0.3	1.8	26	0.2	1.5	30	0.2	1.8

Table 3. Summary of data relative to growth in CWI for the four treatments and the overall average for all four treatments in the 2005-2006 study.

Trait	UN	SD	UG	SD	S	SD	D	SD	Overall average
Infl (N)	16		14		15		6		12.8
Ave Infl Height (mm)	652.1	48.1	826.4	56.3	785.9	52.1	625.7	46.5	722.5
Ave No. Branches	5.3	0.3	5.4	0.4	5.6	0.4	3.2	0.2	4.9
Ave No. Fruits	3.9	0.2	4.4	0.2	5.5	0.3	2.8	0.1	4.2

growing seasons and only six and five tip-down CWI bulbs respectively produced flowers and fruits during the 2005-06 and 2006-07 growing seasons.

Initial bulb weight as a predictor of reproductive success – first experiment (2003-05).—An analysis of covariance (ANCOVA) showed that initial bulb weight was not related to stalk size ($P=0.2115$), the number of side branches on a flowering stalk ($P=0.7647$), or the number of fruit produced ($P=0.5009$) for the 2003-04 growing season. Further, a logistical regression showed that the initial bulb weight could not be used to predict if a bulb would produce a flowering stalk ($P=0.3033$). Lack of any correlation between initial bulb weight and reproductive success further indicates that the method of bulb selection used for this study did not result in any bias in the experimental results. Since initial bulb weight was not a significant predictor of reproductive success, this analysis was not completed for the second experiment (2005-07).

Bulb sprouting pattern.—As the bulbs were removed from the pots at the end of the 2004-05 season, we were particularly interested in examining bulbs that had been planted oriented parallel to the soil surface (S-bulbs, i.e., planted on their side) and bulbs that had been planted upside-down (D-bulbs). We had noted that the plants developing from these bulbs arose at the edges of the pots rather than from the center of the pot where the bulb had been initially planted. It appeared as if the bulbs sprouted and elongated until hitting a surface – in this case the pot wall – and then turned and grew upward until finally emerging from the soil surface. Upon digging up the bulbs, we verified that this was indeed the situation. In contrast, bulbs planted upside down (Fig. 3) seem to have grown downward until hitting the base of the pot, then grew obliquely until apparently hitting the side wall of the pot, and then finally completed an upward growth toward the soil surface. In these cases, no bulb reorientation to gravity occurred within the pots.

Harvest bulb weights – Experiment 1 (2003-05).—Bulb weights were not significantly different among treatments when first planted in 2003 ($P>0.05$). However, bulb weight among treatments when the bulbs were harvested in 2005 did differ in that the control Up–Native (UN) and Up–Greenhouse (UG) bulbs as a group were significantly smaller than the Side–Greenhouse (SG) and Down–Greenhouse (DG) treatment bulbs ($P<0.05$). Note also that average bulb weights in the Up–Greenhouse and Up–Native control groups were significantly less (t-test, $P<0.05$) when harvested in 2005 than those originally planted in 2003, whereas there were no significant differences in such bulb weights for the SG or the DG treatments ($P>0.05$, Table 5). Bulb weights tended to decrease in the UN and UG controls, whereas they generally increased slightly in the SG and DG treatments. Weight loss between seasons averaged more than 35% in the Up-Native (UN) and Up-Greenhouse (UG) controls, whereas weight gain averaged more than 5.5% in the SG and DG treatments.

Harvest bulb weights – Experiment 2 (2005-07).—Bulb weights were not significantly different among treatments ($P>0.05$) when first planted in 2005 and were also not significantly different among treatments when these bulbs were harvested in 2007 ($P<0.05$). However, it

Table 4. Summary of data relative to growth in CWI for the four treatments in the 2006-2007 study with percentage similarity to 2005-2006 for comparison.

Trait	UN	% 03-04	SD	UG	% 03-04	SD	S	% 03-04	SD	D	% 03-04	SD	Overall average	% 03-04
Infl (N)	7	44		9	64		12	80		5	83		8.3	65
Ave. Infl Height (mm)	417.4	64	33.8	654.4	79	29.6	685.0	87	41.5	553.0	88	38	577.5	80
Ave No. Branches	1.7	32	0.2	2.1	34	0.4	1.9	34	0.3	1.4	44	0.1	1.8	37
Ave No. Fruits	5.0	128	0.3	4.3	98	0.3	3.6	65	0.4	2.4	86	0.1	3.6	86

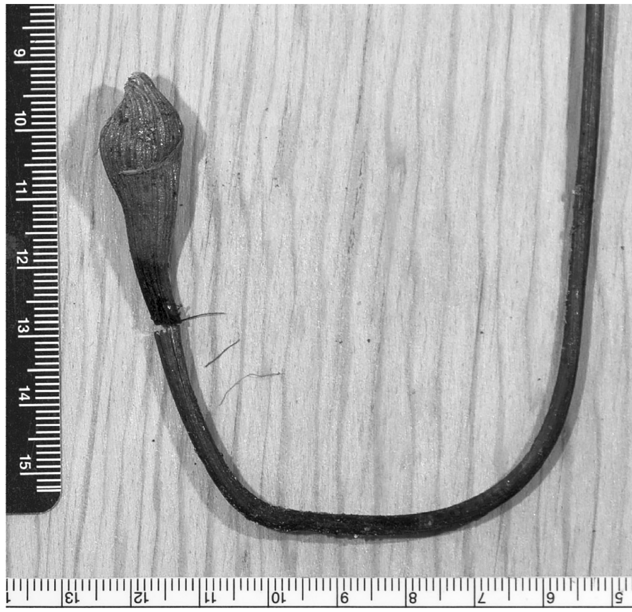

Fig. 3. Growth pattern in bulbs planted upside-down.

is interesting to note that the average weight of the bulbs planted in 2003 was significantly greater than those planted in 2005 ($t=126.21$, $df=78$, $P<0.0001$). The average bulb weight in 2003 was 4.82g, whereas the average bulb weight in 2005 was 2.61g (Table 6).

Reproductive success by treatment during the first two-year study (2003-05).—A comparison of sexual reproductive success between the two growing seasons can be seen in Tables 7 and 8. Note that most bulbs, with the exception of the D treatment, reproduced successfully during the 2003-04 growing season, but there was a substantial reduction in reproductive success in all treatments, with the exception of the D treatment during the 2004-05 growing season.

Discussion

The energetics of plant growth and reproduction is discussed by several authors including Fiedler (1987), Philippi and Seger (1989), and Fenner (1998), Miller, et al. 2004), and Marques and Draper (2012). In our study, CWI bulb orientation at planting in CWI clearly did influence the energy input that affected subsequent growth and reproduction, most notably in the tip-down

Table 5. Comparison of bulb weights for each treatment when bulbs were planted and harvested in 2005 and 2007.

Group	N	Ave. lf. width	SD	Leaf only	Flower stalk	flowers	Mature Fruit	D/D	% Repro
UN	20	12.7	0.1	1	-	-	19	-	95
UG	20	13.2	0.1	1	-	-	19	-	95
S	20	15.5	0.2	3	-	-	17	-	85
D	5	15.2	0.3	3	-	-	4	15	20
Overall Ave. %	81.25	14.2	0.2	7.5	0	0	73.7	18.8	73.75

Table 6. Comparison of the maximum stage attained for growth and reproductive success by treatment for 2003-2005.

Bulb orientation	2005 sample size (N)	Ave. bulb wt. 2005 (g)	SD	Ave. bulb wt. 2007 (g)	SD	2007 sample size (N)	Ave. wt. loss or gain	SD	Range of wt. loss or gain
UN	20	2.43	0.5	3.37	0.9	14	+39%	0.8	−3 to +124%
UG	20	2.67	0.2	2.95	0.4	15	+10%	1.9	−41 to +317%
SG	20	2.69	0.8	3.21	2.1	20	+19%	1.7	−87 to +175%
DG	20	2.66	1.1	2.72	2.3	7	+2%	2.0	−69 to +250%

(D) treatment. Fruit-set for the D treatment was uniformly low, 20% for both years of experiment 1 and 25% for both years of experiment 2.

When all bulbs were removed from the pots at the end of experiment 2 and weighed, it was apparent that the values for the control bulbs (UN and UG) were, in nearly all cases, noticeably lighter in weight than those recorded for the original bulbs planted in 2003. In contrast, over half of the bulbs surviving from the other two treatments (S and D) weighed more than the original bulbs. This may mean that the successful growth and fruit-set in UN and UG bulbs during the first year required substantial energy and resulted in the subsequent formation of smaller bulbs (possessing less stored energy) and in fewer bulbs setting mature fruit during the second season. Bulb weight (as an indicator of stored reserves) and, therefore, reproductive success, thus may partially explain the episodic reproductive success that has been recorded for several species of *Calochortus* (Fiedler 1987; Miller and Douglas 2001); Miller et al. 2004). That is, the bulb dormancy that often follows years of substantial reproduction may be explained, at least in part, by the formation of insufficient energetic reserves to allow for successful reproduction in consecutive years (Fenner 1998; Marques and Draper 2012). However, other factors may also play a significant role in reported cases of apparent synchronized bulb dormancy (dormancy across sites within the species geographic distribution), as has been suggested by Miller et al. (2004).

When bulbs were examined and weighed at the end of experiment 2, the weights were not significantly different from one another. In all treatments including the tip-down (D) bulbs, bulb weight at harvest in 2007 did increase somewhat although not significantly over the weight of the bulbs when initially planted in 2005. Harvested bulb weights in 2007 were similar for the two control treatment bulbs (UG and UN). However, the increases in harvested bulb weights for the tip-down (D) and side-planted bulbs (S) were greater at the end of 2005, than they were in those bulbs harvested at the end of 2007. In contrast to the pattern of bulb weights observed at the end of experiment 1 (2003-05) in which the control plants (UN and UG) decreased an average of over 40% (range +8 to −79%) in their bulb weight, the bulb weights of these two control treatments actually showed an average increase over initial bulb weight of about 25% (range −3 to +317%) during experiment 2 (2005-07). The decreased bulb weight during 2003-05 was in

Table 7. Comparison of the maximum stage attained for growth and reproductive success by treatment for 2005-2007.

Bulb orientation	2005 sample size (N)	Ave. bulb wt. 2005 (g)	SD	Ave. bulb wt. 2007 (g)	SD	2007 sample size (N)	Ave. wt. loss or gain	SD	Range of wt. loss or gain
UN	18	9.6	0.1	10	1	-	7	2	35
UG	19	8.1	0.2	9	2	1	7	1	35
S	20	9.3	0.1	7	4	1	8	-	40
D	13	9.9	0.3	6	3	-	4	7	20
Overall Ave. %	87.5	9.2	0.2	40	12.5	2.5	32.5	12.5	32.5

Table 8. Comparison of the maximum stage attained for growth and reproductive success by treatment for 2005-2007.

Group	N	Ave. lf. width	Leaf only	Flower stalk	Flowers	Mature fruit	D/D	% Repro
UN	18	9.6	10	1	-	7	2	35
UG	19	8.1	9	2	1	7	1	35
S	20	9.3	7	4	1	8	-	40
D	13	9.9	6	3	-	4	7	20
Overall Ave. %	87.5	9.2	40	12.5	2.5	32.5	12.5	32.5

sharp contrast to the manipulated bulbs (S and D), which did not have the reproductive success of the two controls during (2003) the first growing season (95% for both controls – UN and UG, 85% for S and 20 % for D), but also did not suffer nearly the subsequent bulb weight decrease of the controls (in fact, manipulated bulbs experienced an average bulb weight gain of between 5.7% (D) and 9.5% (S) during the first two years of that study – 2003-05). Even the difference in final bulb weights between these two treatments may be the result of their relative expenditure of energy during the two years of the study for growth and reproduction. An examination of the growth patterns of these two treatments during the first two-years of this study (2003-05) would seem to support this conclusion since the amount of growth required to break the soil surface and establish an initial basal leaf (required for photosynthetic activity) by the D bulbs versus the S bulbs appeared to be substantial.

During experiment 2 (2005-07) bulb weights of the two control treatments (UN and UG), as well as the two manipulated treatments (S and D), actually showed an increase in average bulb weight (of about 17%) with UN showing the largest average weight gain of 39%, compared to 10% for UG, 19% for S, and 2% for D. It may be that the planting of smaller bulbs in 2005 at the beginning of the second two-year study produced a greater tendency for these smaller bulbs to put less energy into reproduction and more into carbohydrate storage in the bulb (Fielder, 1987). It would appear that the growth patterns exhibited by these two treatments (S and D) would necessitate a greater energy expenditure just to break the soil surface and begin to produce energy by photosynthetic activity in the basal leaf. However, during both two-year studies (2003-05 and 2005-07), the greatest average weight increase in S and D bulbs at harvest was 2% in D bulbs in 2007 and 19% in S bulbs in that same year.

Sexual reproductive success during experiment 1 (2003-05), as measured by the percentage of bulbs forming mature fruit, dropped off substantially for most plants during the second season (2005) with values of 30% for UN bulbs, and 40% for UG and S bulbs. Only the D bulbs were able to maintain the same, albeit low, level of reproductive output (20%) during the two years. A similar pattern of reproductive success was observed for experiment 2 (2005-07) in which 45% of the UG bulbs, 60% of the S bulbs and 25% of the D and UN bulbs produced fruit. Only the D bulbs maintained the same level of reproductive output between the two years even though the number of successfully reproducing plants was much smaller than that found in the other three treatments. All other treatments showed a decline in reproductive output.

Implications for management strategies indicate that bulb death and/or dormancy are far greater in the D treatment (upside down polarity) during both experiments than in the controls (UN and UG) or the S treatment, although this condition also did appear to increase in the UN bulbs during the 2007 of experiment 2. However, the treatment bulbs that did survive (both S and D) were able during both studies, on average, to store up a greater mass of photosynthate than did either of the control groups. This latter unexpected observation may possibly result in greater long-term survival and establishment of bulbs planted with these orientations in new populations in the mitigation areas, but this clearly requires examination over a longer study period before recommendations can be made. A consistent pattern of reduced survival of all treatment bulbs became apparent in the second year of each of the two sets of replicated studies. It may be that *Calochortus* bulbs do not do well when kept longer than one year in pots under controlled conditions.

A number of aspects of this study would seem to warrant further examination. For example, D bulbs that did emerge from dormancy during both years of the first two-year study began doing so between one and two months after the tip-up controls (UN and UG) and 2-3 weeks after the side-planted group. Maximum emergence of D bulbs was five plants in 2003-04 and 13 in 2004-05. During the second two-year study (2005-07), D bulbs seemed to emerge from dormancy faster than in the first two-year study (2003-04).

Tip-up controls planted in native soil (UN) presented the most complex patterns of response. Reproductive success, as measured by fruit-set, varied among treatments during the four years of study. Fruit-set in plants from UN bulbs was low in first year of each experiment (2003-04 and 2005-06), but improved in 2004-05 and 2006-07 when UN plants produced the highest number of fruits per flowering stalk. Fruit-set in plants from D bulbs was usually among the lowest in each of the four years of this study (2003-04, 2004-05, 2005-06, and 2006-07). Plants from UG and S bulbs varied noticeably in reproductive output, but did have the highest reproductive output in first year of each two-year study (2003-04 and 2005-06).

As previously noted, low fruit-set in plants from D bulbs makes sense from an energetic standpoint, since more energy would have to be devoted to the growth of the stem from the bulb to the soil surface than in the other three treatments. This energetic constraint seems to be corroborated by the low survival rate of the D bulbs at the end of each two-year study (in 2005 and 2007), when their survival rate was between 35% and 65% of that of the other three groups (UN, UG, and S bulbs). However, the variation in fruit-set in the other three treatments is more difficult to explain and requires further investigation. Further, at present, the reasons for the various differences in fruit-set with time in the ground (first and third years versus the second and fourth years of this four-year study) for the plants derived from UN and D bulbs needs further examination.

From the above summaries, it can be seen that bulb orientation at planting did have an influence on both qualitative and quantitative aspects of growth, i.e., on the timing of some processes and on the size and/or numbers produced by these processes, but the pattern that emerged in each experiment during the second year (2004-05 and 2006-07) was dissimilar in many ways from that found during the first growing season (2003-04 and 2005-06). The only consistent pattern to emerge was that many fewer bulbs planted in the upside down (D) orientation survived and/or set mature fruit each year than in the other treatments. Therefore, care should be given to ensure that bulb orientation during replanting of salvaged mitigation bulbs is accomplished with the bulbs planted in the proper polarity (growing tip upright). For the other parameters (e.g., bud formation, flower opening, and fruit set), planting orientation did not appear to be the major factor influencing the timing of the process (ambient/soil temperatures, soil moisture levels, and/or photoperiod would seem to be more likely cues).

Several of the quantitative effects may also be a consequence of carbohydrate availability limitations reflected in the limited number of D bulbs that emerged during the first season as compared to the second season (5 versus 13 in 2003-04 versus 2004-05). As stated above, the D treatment experienced the largest bulb mortality of all treatments during the course of each of the two-year studies. It is interesting to note, however, that surviving D bulbs actually registered an average weight increase of 5.7% in the second year bulbs during the first two-year study (end of 2005), which was second only to the 9.5% weight increase seen in comparable S bulbs. However, although the surviving D bulbs did experience an average weight gain of 2% during the second two-year study (when the bulbs were harvested in 2007), all three other treatments experienced a greater average bulb weight gain (UN=39%, UG=10%, and

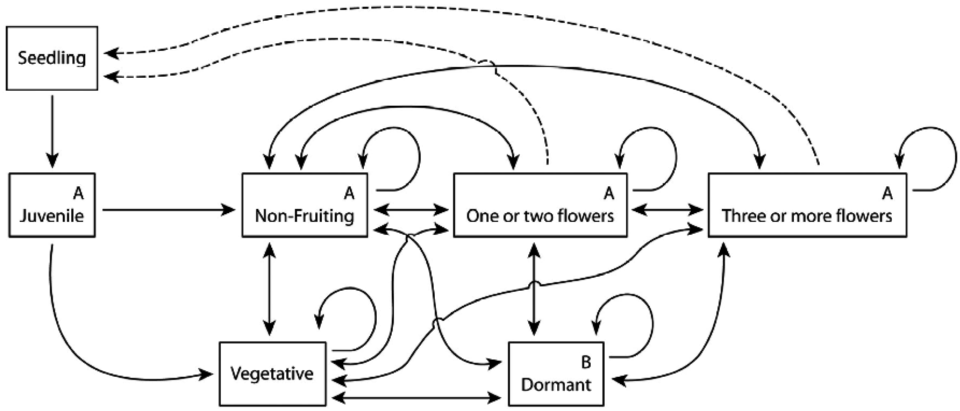

Fig. 4. Model for growth and development in CWI.

S = 19%). It is unknown why the results for bulb weights at the end of each of the two-year studies is so different, but it may be related to the differences in average bulb weights at initial planting during 2003 and 2005 of each study. The significantly smaller size of bulbs utilized during the second two-year study (starting in 2005) may have contributed to this difference, since smaller bulbs tend to devote much of their photosynthate production to increasing bulb reserves to a point that ensures a greater probability of a successful reproduction event (Fiedler 1987; Philippi and Seger 1989; Fenner 1998; Worley and Harder 1999).

Judging from the current data, it is quite possible that bulb orientation at planting may not be a significant factor in the long-term survival of individual bulbs. However, from the standpoint of bulb population mortality rate, it would be better to plant the bulbs in either an upright or, at least, a sideways orientation and avoid, if possible, an up-side-down orientation. An additional aspect of interest arising from our four-years of study (2003-07) was the degree of dormancy seen in bulbs that were initially planted in the up-side-down orientation. It would seem that this increased dormancy could create problems when trying to assess the effectiveness of bulb transplantation as part of the mitigation process, in that bulb survival could potentially be greatly underestimated if monitoring of the project to determine transplantation success rate is limited to one year. It is additionally apparent from our study and from the literature (see Fiedler 1987 and Miller et al. 2004 as examples) that population densities of naturally occurring geophytes, such as CWI, may be greatly underestimated due to dormancy episodes that can last a single year or more. It is currently unknown which internal or external factors may induce such dormancy in natural populations, although Miller et al. (2004) found that such episodes were apparently synchronized across sites within the geographic distribution of a given species.

As an aid to further investigative efforts, the data collected in this study and data from the final report to LSA Associates 2008 for vegetative data not reported here were used to develop a preliminary model for growth and development in CWI (Fig. 4). Plants producing greater than three flowers are much more likely to set mature fruit than are ones with fewer flowers. This is probably related to the availability of greater photosynthate reserves stored in the bulbs from which the former plants normally arise. If the photosynthate reserves are reduced for any reason, the bulbs may produce a smaller plant that: 1) has only one or two flowers; 2) may be non-fruiting; 3) may be strictly vegetative; or 4) may even go dormant for one or more years. Bulbs may produce new smaller bulbs asexually if an external stimulus, such as some type of stress, initiates the process (Fiedler 1987). Some bulbs may even have a genetic predisposition toward this type of cloning. Each stage may remain in that condition for a year or more (Fiedler 1987).

Conclusions

The data from this study do indicate that: 1) bulb planting orientation does influence survival, growth, and reproduction and 2) proper bulb planting polarity (orientation) should be an important consideration in any transplantation of this or any sensitive bulb producing plant species for mitigation purposes. Based on our results, we predict negative effects if the shoot apical meristems of salvaged bulbs are not carefully planted in a normal tip-up orientation during transplantation. We predict that negative effects would include one or more of the following: 1) abnormal bulb dormancy or death; 2) abnormal energy-wasting subterranean growth patterns; and 3) suppressed sexual reproduction, at least in the short term.

Figure 3 illustrates what happens when D or S bulbs turn upward after coming in contact with the pots. It appears there may be a lack of negative gravitropism in this species. We recommend future studies should investigate this possibility by planting D and S bulbs in the ground and following what then occurs. One would expect negative gravitropism to affect the growth of the shoot, but it may take longer to occur and further deplete the energy reserves of the bulb reducing the survival and reproductive output of these bulbs. As an aid to further investigative efforts, the data collected in this study were used to develop a preliminary model for growth and development in bulb producing plants.

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